This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

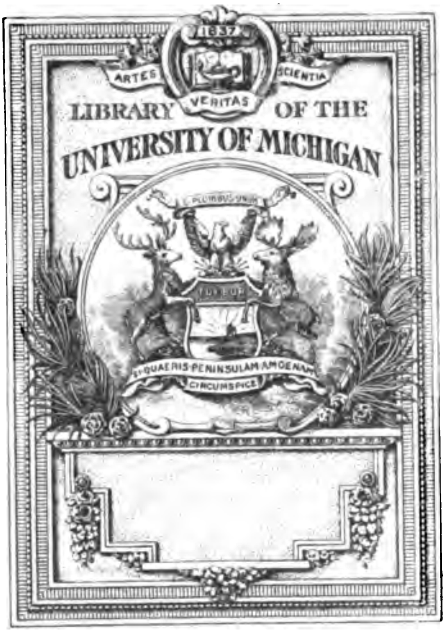
+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

A 490241



LIBRARY OF THE
UNIVERSITY OF MICHIGAN

AS
36
.B916
M7

285.c

BRYN MAWR COLLEGE MONOGRAPHS

REPRINT SERIES, Vol. V.

CONTRIBUTIONS FROM THE BIOLOGICAL LABORATORY

BRYN MAWR, PENNA., U. S. A.
April 1905

Table of Contents

I. Polarity and Regeneration in Plants. By T. H. Morgan.
(*Reprinted from the Bulletin of the Torrey Botanical Club, 31, April, 1904.*)

II. On the Germ Cells and the Embryology of Planaria simplicissima. By N. M. Stevens.
(*Reprinted from the Proceedings of the Academy of Natural Sciences, Philadelphia, April, 1904.*)

III. Self-Fertilization induced by Artificial Means. By T. H. Morgan.
(*Reprinted from the Journal of Experimental Zoölogy, Vol. I, No. 1, May, 1904.*)

IV. Notes on Regeneration in Tubularia crocea. By Helen Dean King.
(*Reprinted from the Biological Bulletin, Vol. VI, No. 6, May, 1904.*)

V. The Regeneration of the First Leg of the Crayfish. By Margaret A. Reed.
(*Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XVIII, No. 3, August, 1904.*)

VI. Closure of Longitudinally split Tubularia Stems. By Alice M. Boring.
(*Reprinted from the Biological Bulletin, Vol. VII, No. 3, August, 1904.*)

VII. Regeneration in the Planarian, Phagocata gracilis. By T. H. Morgan and Alice E. Schiedt.
(*Reprinted from the Biological Bulletin, Vol. VII, No. 3, August, 1904.*)

VIII. An Examination of the Problems of Physiological Polarity and of Electrical Polarity in the Earthworm. By T. H. Morgan and Abigail C. Dimon.
(*Reprinted from the Journal of Experimental Zoölogy, Vol. I, No. 2, August, 1904.*)

IX. The Regeneration of a Heteromorphic Tail in Allolobophora foetida. By Abigail Camp Dimon.
(*Reprinted from the Journal of Experimental Zoölogy, Vol. I, No. 2, August, 1904.*)

147466

X. A Contribution to the Embryology of Hylodes martinicencis. By Lilian V. Sampson.
(Reprinted from the American Journal of Anatomy, Vol. III, No. 4, September, 1904.)

XI. The Relation between Normal and Abnormal Development of the Embryo of the Frog (III), as Determined by some Abnormal Forms of Development. By T. H. Morgan.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XVIII, No. 4, October, 1904.)

XII. Results of Injuries to the Blastopore Region of the Frog's Embryo. By Anne Hampton Todd.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XVIII, No. 4, October, 1904.)

XIII. The Relation between Normal and Abnormal Development (IV), as Determined by Roux's Experiment of Injuring the First Formed Blastomeres of the Frog's Egg. By T. H. Morgan and Ellen Torelle.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XVIII, No. 4, October, 1904.)

XIV. The Formation of the Interior Cells in the Segmentation of the Frog's Egg. By Margaret Reed.
(Reprinted from the Biological Bulletin, Vol. VIII, No. 3, February, 1905.)

XV. Further Studies on the Ovogenesis of Sagitta. By N. M. Stevens.
(Reprinted from the Zoolog. Jahrbücher, Vol. XXI, Abt. für Morph., February, 1905.)

XVI. Experiments on Polarity in Tubularia. By T. H. Morgan and N. M. Stevens.
(Reprinted from the Journal of Experimental Zoölogy, Vol. I, No. 4, February, 1905.)

XVII. An Attempt to Analyze the Phenomena of Polarity in Tubularia. By T. H. Morgan.
(Reprinted from the Journal of Experimental Zoölogy, Vol. I, No. 4, February, 1905.)

XVIII. The Relation between Normal and Abnormal Development of the Embryo of the Frog (V), as Determined by the Removal of the Upper Blastomeres of the Frog's Egg. By T. H. Morgan.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XIX, No. 1, February, 1905.)

XIX. Experimental Studies on the Eye of the Frog's Embryo. By Helen Dean King.
(Reprinted from the Archiv für Entwickelungsmechanik der Organismen, Vol. XIX, No. 1, February, 1905.)

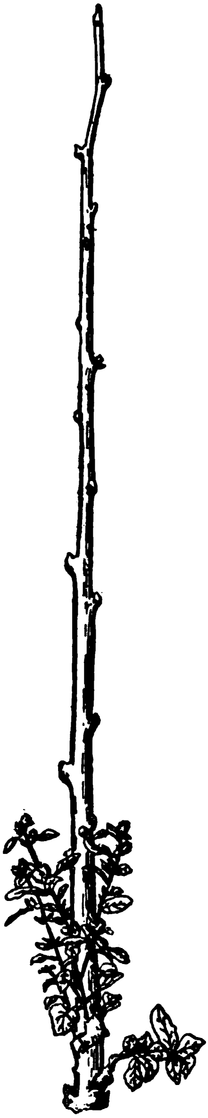
[From the BULLETIN OF THE TORREY BOTANICAL CLUB, 31: 227-230. April, 1904.]

Polarity and regeneration in plants

T. H. MORGAN

The earlier and greater development of the buds that stand at the distal end of a piece of a willow than of those that stand nearer to the base of the piece bears a certain general resemblance to the phenomenon of polarity in animals, and has led, in fact, to the use of the same word for both processes. This comparison needs, I think, to be more critically examined. During the past summer I have kept pieces of several plants, from which the leaves were removed, in a moist chamber with the lower end of the pieces in water, and have watched the development of the buds. In most cases, as in the willow, the more distal, *though not necessarily the most distal*, buds are the first to develop, and it could be easily seen that those that unfolded first were, as a rule, the largest and most advanced buds present on the piece when it was removed from the plant. In other words, the relative strength of the buds determines which develop first, and it seems most plausible that in consequence of this development the other buds might be kept from unfolding because those that got the start used up all the available food substances that were present, or were being manufactured in the piece. It appears, therefore, that the result is not so much the outcome of the polarity of the piece, acting at the time of regeneration, as of preexisting conditions in the piece at the time of its removal from the plant. These determine which buds shall be the first to unfold. Whether or not this difference in the condition of the buds of the original piece has itself been regulated by polar relations in the growing point is a question for further consideration, but in any case it is one that does not involve the immediate question of the regeneration of the isolated piece.

In one of the plants that I examined, the proximal and not the distal buds of the piece were the first to develop. It is this case that I wish more especially to discuss in connection with the problem of polarity in plants. The plant was the common burdock, *Arctium Lappa* (*Lappa officinalis*). The leaves and the lateral branches were cut off from half a dozen vigorous plants, and the denuded stalk was left standing in place in connection with its original roots. In the course of about ten days new buds began to grow out near the base of the stalk. They unfolded rapidly and at the end of about two weeks had reached the condition shown in the figure. The new shoots arose in or near the angles between the main stem and the lateral branches (which had been cut off). These branches stand in the axils of the lower leaves. No shoots at all appeared in the upper regions of the stem, although the latter remained green and in good condition. It may appear that the results in this case are connected with the attachment of the stem to the old roots. The fact that large branches arise near the base of the main stem may be interpreted to mean that these parts receive a large share of the substances that come up from the roots. In order to see if this suggestion had any value I cut off other stems from their roots, stripped them of their leaves and branches, and placed the lower ends in dishes of water. In these cases also the lower buds alone unfolded and none appeared in the upper parts of the stem.



To determine whether the result is due to the better development of the buds at the base rather than due to the movement towards the base of food or of so-called formative substances, I cut a few of the stems into three pieces. In those in which any development took place this occurred in the basal pieces, and in one case in the lower part of the second piece as well, but no buds developed in the distal pieces, although to all appearances these remained in good condition. Whether these distal pieces would after a much longer time (I kept them nearly three weeks) have developed buds, I do not know.

This last experiment, while not satisfactory in all respects, yet suffices to show that the development of the basal buds in the long pieces is not due to the polarity localizing, as it were, the development at the base, nor to the flow of substances downward, but is due to the stronger buds being present in the basal region.

These results recall the cases of *Lilium candidum* and *Lachenalia luteola.* These plants do not set seed, but produce bulblets at the base. This formation of basal bulblets is attributed by Goebel to the flow of food substances in the plants towards the base which causes the bulblets to develop in this region, and at the same time deprives the seeds of the necessary material for their development. The explanation appears to me to be exactly the reverse. The buds that give rise to the bulblets in these plants are so vigorous that they utilize all of the food substances that are present, and thus deprive the seeds of food material that they might possibly make use of if the bulbs did not develop. It is not, I think, the flow of food substances downwards that causes the bulblets at the base to develop, but the vigorous bulblets in this region draw into themselves so much of the available food substances that not enough is left for the seeds.

It might be claimed in the case of the burdock, that when the stalk, deprived of its leaves, is left attached to the old roots, material from the roots rising up into the stem will affect the basal buds first; or it might be claimed that since there are large fibrovascular bundles that go to the basal nodes these bring to this region materials from the roots that cause the buds to develop, but that this is not the real explanation was shown above by the experiment of removing the stem from the roots. The result appears to be due rather to the more vigorous condition of the basal buds. Whether, as I have said, this condition of the plant is itself to be thought of as ultimately the outcome of its polarity, is a question that I do not think we can profitably discuss as yet. If it is, then the polarity in the growing point has already acted and determined the relative development of the buds in the different regions. When the piece containing these buds is removed, their further development is first determined by the stage that they are already in, or by their greater vigor, which may, in most cases, mean the same thing. In the second place certain buds having gotten a start use up all or most of the available food materials and thus check the further development of the other buds.

In the light of these facts and conclusions certain of the statements that I made in my book on "Regeneration" in regard to the cause of the development of the apical buds in a piece of the willow must be recast. The development of the apical buds of the willow, and of other similar plants, and of the basal buds in the burdock appear both to be due, not to a dynamic relation (polarity) between the two ends of the piece, but to a static condition already existing in the piece before its removal, namely, the relative state of development of its buds.

From this point of view Sachs' theory of formative stuffs plays no directive part in the regeneration of pieces of the plants. The presence of food stuffs enters into the problem only in so far as certain parts are supposed to be able to draw on that which is present, while other parts (the less developed buds) are not so able to make use of the common supply. The flow of these food stuffs through the plant appears from this point of view not to be due to the stuffs tending to flow of themselves in certain directions, or as the result of the action of some outside agent, as gravity, but their flow may be simply a question of diffusion from those places where they exist in larger amounts to other places where there is not so much of the substance present. If the more vigorous and somewhat older parts can make use of this material more rapidly than can the less well-developed parts, there will be a steady flow of soluble food substances towards the growing parts, because in these regions the material is being more rapidly used up, and hence the region is relatively poorer in these materials. The flow is then a purely physical problem. This assumption is, of course, not different from that usually employed by botanists to account for the flow of soluble substances from one part of a plant to other parts.

It appears, therefore, that polarity in the plant is not the cause of the flow of substances through the plant, as Goebel seems to imply in certain parts of a recent article,* nor does polarity appear to regulate the development of certain buds and hold others in check.† Possibly some such factor may determine in the growing regions of the plant the relative rate of development of certain buds, but even this is not certain and remains to be further examined.

BRYN MAWR COLLEGE.

---

*Goebel. Bull. Torrey Club, 30: 197–205. 1903. Also Biolog. Centralbl. 22: 385–397, 417–438, 481–505. 1902.

† Morgan. Bull. Torrey Club, 30: 206–213. 1903.

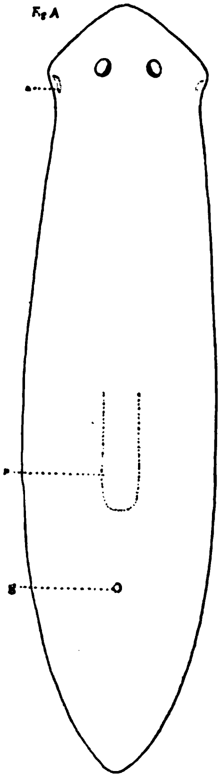
ON THE GERM CELLS AND THE EMBRYOLOGY OF PLANARIA SIMPLISSIMA.

BY N. M. STEVENS.

This planarian, which is found in small streams about Bryn Mawr, was identified provisionally in 1900 by Woodworth as *Planaria lugubris*, and has since figured under that name in several of Prof. T. H. Morgan's papers on regeneration; also in my "Notes on Regeneration in *Planaria lugubris*" (Stevens, '01).

On looking up the European species (*P. lugubris*) as described and figured by Schmidt ('59, Pl. III, figs. 5 and 6), and by Kennel ('79, Pl. VII, fig. 8), I felt sure that Woodworth must have been mistaken as to the species; but I was unable to find any correct description or figures, either of the external characters of the animal or of its reproductive organs, and I was inclined to call it a new species. In September, 1903, after this paper was written, I came across an article by Curtis ('00) on the reproductive organs of *Planaria simplissima* n. sp. The reproductive organs of this species were so strikingly like those of the form on which I had been working that, although there was considerable difference in form, size and color, I was convinced that the two worms must be closely related, if not local varieties of the same species.

In answer to my inquiries about *Planaria simplissima*, Prof. Curtis has recently written me that after studying specimens sent to him by Prof. Morgan from Bryn Mawr, and sectioning others of the same species found near Baltimore in 1900 and 1901, he concluded that the Williamstown form, *P. simplissima* (fig. B), and the Bryn Mawr form *P.* (*lugubris*), (fig. A) belonged to the same species. Prof. Curtis desires me to state that his description of the external characters of *P. simplissima* was made from fixed material, living specimens not being accessible when he discovered that he was dealing with a new species. Later observations on living specimens from Williamstown made it apparent that his description was at fault, especially with regard to the lateral cephalic appendages which are more marked than was evident in fixed material. A careful sketch, made at this time from the living animal and sent to me with his letter, is a good representation of a young specimen of the Bryn Mawr form (figs. A and B)



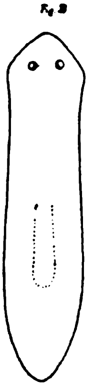


Fig. A. Outline sketch of a large mature specimen of *Planaria simplissima*. *a*=gray sensory area on the lateral auricular appendage. *p*=pharynx. *g*=genital opening.

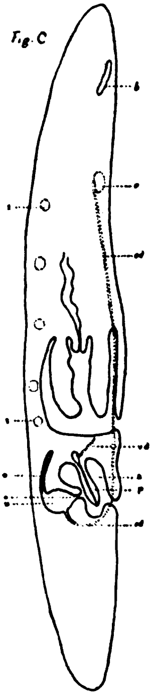
Fig. B. Outline drawing from Curtis's sketch of a small specimen from Williamstown.

and, I think, leaves no doubt that we have the same species, which may be described as follows:

Planaria simplissima Curtis.

Length of mature specimens 7–15 mm.; breadth 2–4 mm. Color a nearly uniform seal-brown (occasionally grayish) with an inconspicuous gray area on each cephalic appendage. Eyes gray with a crescent of black pigment on the median side. Both anterior and posterior ends blunt. Lateral cephalic appendages blunt and inconspicuous as compared with *P. maculata*. Body thick as compared with *P. maculata*. Pharynx just posterior to the middle point of the longitudinal axis of the worm. Ovaries two, ventral, somewhat lobed, and situated about half-way from the anterior end of the animal to the pharynx.

Testes four or five on each side, unpaired, dorsal, and irregularly distributed from the region of the ovaries to the posterior end of the pharynx (figs. C and D). Penis long and slender, not filling the antrum. Uterus consisting of a chamber lined with glandular epithelium, dorsal to the antrum, and with an anterior prolongation in the form of a narrow ciliated tube with no enlargement at its anterior end. Vasa deferentia two, opening separately into the anterior enlargement of the lumen of the penis. Oviducts two, ventral and parallel with the nerve-cords, uniting before entering the uterus (figs. C and D). Vitellaria extending from the region of the ovaries to the posterior extremity of the animal.



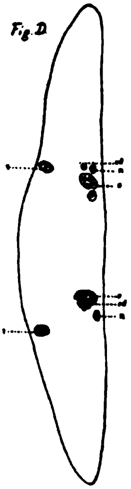


Fig. C. Median longitudinal section of *Planaria simplissima* showing reproductive organs. Parts out of the plane of the section are shown in dotted lines. *a*=antrum. *b*=brain. *o*=ovary. *od*=oviduct. *p*=penis. *t*=testis. *u*=uterus. *vd*=vas deferens of one side. *x*=ciliated tube opening into uterus. *v*=vagina.

Fig. D. Reconstruction from several cross-sections showing ovaries (*o*), oviducts (*od*), nerve cords (*n*) and testes (*t*).

Found on the under side of stones and leaves along the margin of small streams.

The original object of this paper was a discussion of the reproductive organs, ovogenesis, spermatogenesis and embryological development of *Planaria* (*lugubris*). The discovery that it is not *P. lugubris* but *P. simplissima* Curtis renders further discussion of the reproductive organs unnecessary, and I shall therefore confine my attention to a study of the germ-cells and the embryology of this species, which presents some peculiarities not fully described by Ijima ('84) and Hallez ('79).

Fertilization.—Copulation has not been observed in this species, but there is every reason for supposing that it occurs, for spermatozoa are found only in the vasa deferentia, the lumen of the penis, the uterus and the oviduct. In nearly every specimen the anterior end of the oviduct is crowded with spermatozoa (Pl. XIII, fig. 1, *od*), while only occasionally one is found in the posterior part of the duct or in the uterus. The spermatozoa are never found among the oöcytes in the ovary, and it is probable that each egg is fertilized as it enters the oviduct, for the spermatozoon is always found in the eggs of a forming capsule, and no spermatozoa are found among the eggs and yolk.

I should therefore agree with Ijima in regarding the uterus as a gland for forming the cocoon shell, and not as the place where fertilization occurs (Hallez), or as a receptaculum seminis (Kennel).

When an egg-capsule is forming, the antrum, uterus and the tube *x* (fig. C) are all thrown into one chamber, which is filled with eggs and yolk-cells, the penis being pushed back against the anterior wall of the antrum (fig. E) and the antrum being separated from the pharynx-chamber by so thin a layer of tissue that it is often broken through in fixed specimens, and yolk-cells are found in the pharynx-chamber.

Fig. E.

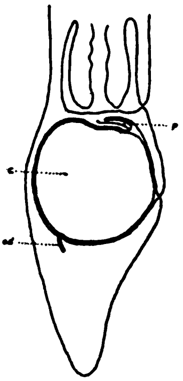


Fig. E. Median longitudinal section through an individual containing an egg-capsule (*c*). *od*= oviduct. *p*= penis

Ovogenesis.—The early stages in the development of the oöcytes evidently should be studied in the summer after laying-time, for the ovaries are practically unchanged in appearance from October to laying-time in April. Figure 1, drawn from a section cut in November, shows nearly all of the oöcytes in the same condition as in sections containing the first maturation-spindle (cut in April and May). The cytoplasm of the oöcytes stains deeply with hæmatoxylin and contains here and there a yolk-granule in a vacuole (fig. 2*a*, *y*). The nucleus is very large and shows but little stainable chromatin, and that in the form of fine granules on threads of linin. The large nucleolus, which stains deeply with orange, contains one or more vacuoles. As in my previous work on the histology of planarians (Stevens, '02), the best results were obtained by fixing the material with sublimate-acetic and staining with Delafield's hæmatoxylin and orange.

The first maturation-spindle is found in the ovary about twenty-four hours before laying. In an equatorial stage the spindle is near the centre of the egg. The asters are very large, but there is no evidence of centrosome or sphere. The chromosomes are V-shaped, and split longitudinally, giving V-shaped daughter chromosomes, as in figs. 3*a* and 3*b*. Only four specimens in this stage were obtained out of a large number sectioned; and of these, three had either 3 chromosomes in an equatorial plate (figs. 2*a* and 2*b*) or 6 daughter chromosomes (figs. 3*a* and 3*b*), and one had 4 in the equatorial plate (fig. 4). Time and material were lacking to trace the egg from the ovary to the uterus, after it was ascertained that an interval of about twenty-four hours occurred between the formation of the first and second polar bodies.

By removing the capsule before the shell is formed and staining with Schneider's aceto-carmine, the second maturation-division can be more advantageously studied than in sections. Figs. 8–11 were made from such preparations; figs. 5*a*, 5*b* and 6, from sections. In only two cases was the first polar body observed (figs. 6 and 8), and it seems probable that it is usually lost as the egg passes down the oviduct. The number of egg-chromosomes is 3 in most cases. In two eggs from the same capsule the number was 6 (figs. 9 and 10), and in a few others 4 and 5 were observed, indicating that, as in *Ascaris megalocephala* and *Echinus microtuberculatus*, there may be two forms which occasionally interbreed, one having twice as many chromosomes as the other.

Thus an egg having 6 chromosomes fertilized by a spermatozoon having 3 would give an individual having 9 somatic chromosomes and probably 5 chromosomes in germ-cells after reduction. Union of germ-cells having 3 and 5 chromosomes respectively would result in an individual having 8 chromosomes in somatic cells, and 4 in oöcytes and spermatozoa.

Figure 8 shows an egg in which there was no doubt about the number 3 in the first polar body (p^1), and at the poles of the second maturation-spindle (p^2 and e). Figure 7 is a somatic cell containing 6 chromosomes. Six have also been counted several times in the first segmentation-division. That the second maturation-division of the chromosomes is longitudinal like the first one is evident from the form of the chromosomes and from the pairs seen at *a* and *b* in fig. 10. Figure 11 shows the second polar body separating from the egg.

Spermatogenesis.—As in the case of the ovaries, the testes should be studied in summer after laying-time, in order to follow the development of the spermatogonia, but occasional divisions of spermatogonia and both spermatocyte-divisions may be observed in material preserved at any time during the autumn and winter. My best material was fixed about the first of December. Pl. XV, fig. 15, shows a part of the section of a testis which contained dividing spermatogonia (*a*), both maturation-divisions (*e* and *f*), spermatids in all stages (*g*, *h*, *i*, *k*, *l*), and ripe spermatozoa. In this animal the number of chromosomes in the maturation-divisions was 4, in the spermatogonia 8. In several others only 3 were found in the spermatocytes (figs. 20 and 22). Various phases of the first maturation-division are shown in figs. 16–21 and of the second in figs. 22 and 23. The form of the chromosomes in all phases of both divisions is the same, a Y-shape, easily distinguishable from the V-shaped and U-shaped chromosomes of the spermatogonia and somatic cells. There is no evidence of a transverse, or reducing, division. In an anaphase (figs. 20 and 22), each daughter chromosome appears to be drawn toward the pole of the spindle by a single fiber attached to the stem of the Y. The spindle is composed of very few fibers, and neither centrosomes nor asters have been demonstrated. The spermatocytes before division appear as in fig. 15, *d*, and nothing corresponding to the synapsis stage described by various authors has been found. The spermatogonia in both resting and division-stages closely resemble the so-called embryonic or parenchyma cells which are scattered through the planarian body and play a conspicuous rôle in regeneration (fig. 15, *a*).

Figs. 24–32 show various stages in the development of the spermatozoön. The nucleus of the spermatid contracts, forming a small ball of nuclear material which stains deeply and uniformly (figs. 24–27, *a*). This concentrated nucleus gradually elongates (figs. 27, *b*–29), and finally leaves the cytoplasm tail first (figs. 15 and 29). Many empty spermatid cells are shown in fig. 15, *n*. The spermatozoön appears to be formed wholly from the nucleus of the spermatid, and stains like chromatin throughout. The spermatozoa in the oviduct near the ovary have a knob-like appendage near the anterior end (fig. 32).

This appears to be a late development, as it is not found on the spermatozoön in the testes, vasa deferentia, or lumen of the penis.

Embryological Development.—As stated above, the first maturation-division of the egg occurs in the ovary; fertilization probably takes place in the oviduct; and the second maturation-division is found in the forming capsules. The eggs of capsules just laid always show the two pronuclei with very large nucleoli, as in Pl. XIII, fig. 12, and a few hours later the pronuclei are fused as in fig. 13, but the two nucleoli are distinct. The development of the eggs during the first day can be best studied in aceto-carmine. Sections of these and of older capsules may be obtained by piercing the shell with a needle and fixing in sublimate-acetic. The shell must be removed before embedding. The rate of development varies greatly in different capsules, and even among the eggs of the same capsule. Laying occurs in the morning from daylight to ten o'clock. In one case the first cleavage-spindle was found at 10.30 A.M. in one egg of a capsule, in which all the others showed the pronuclei not fused. In other capsules eggs containing the pronuclei were found as late as 5 P.M. Two, four and eight-celled stages were also occasionally found late in the afternoon. Figure 14, *a*, was from an egg stained with aceto-carmine at 4.30 P.M. There were 6 chromosomes at each pole, as shown in fig. 14, *b*, obtained by focussing down on one end of the spindle. As in the maturation-divisions, neither centrosome nor sphere could be demonstrated.

The peculiar positions taken by the blastomeres in 2, 4 and 8-celled stages is shown in Pl. XIII, figs. 33–36. Fig. 35 is a reconstruction from five successive sections. Fig. 36 is a section of a 32-celled stage in which the yolk-cells near the group of blastomeres have begun to break down in the region *x*............*x*. The next stage (Pl. XV, fig. 37) shows a section of an embryo, consisting of a syncitial yolk-mass (y^1), distinct from the surrounding yolk-cells and disintegrated yolk-material. The group of blastomeres is always irregular in form and eccentrically situated, coming to the surface on one side of the yolk-mass. Some of the blastomeres soon begin to wander through the syncitium, and may be found dividing at any point. A section of a capsule at this stage frequently shows sections of three or four such embryos. The embryonic yolk-mass gradually increases in size, as may be seen by comparing figs. 37–40, all drawn with the same magnification. The embryo is partly or wholly surrounded by a region of disintegrated yolk-cells (fig. 37, *a*), from which material for the embryonic syncitium is evidently drawn. In some cases whole yolk-cells appear to be taken into the syncitium in amœboid fashion.

In fig. 38, p^1, the first cells of the embryonic pharynx are distinguished from the surrounding blastomeres by their different staining qualities. Figs. 39–41 show the characteristic structure of such a pharynx which is well developed, but not yet functional. Fig. 40 is a median vertical-section through the pharynx, fig. 39 a median cross-section through the central cells (*b*), and fig. 41 a cross-section through the four inner cells (*d*). The cells which surround the lumen of the pharynx are twelve in number—four somewhat flattened surface cells (*a*), four cylindrical central cells (*b*), and four nearly spherical inner cells (*d*). Metschnikoff ('83) suggests that the latter group of four cells, supposed by some to represent the primary endoderm, may serve as a valve to prevent the escape of yolk-cells. The central cells are surrounded by a considerable number of smaller cells radially arranged and supposed to be muscle-cells serving to open the pharynx. Figs. 39 and 40 also show wandering blastomeres in all parts of the syncitial yolk-material of the embryo. A few of these are flattened to form a partial epithelium. Fig. 42 is a section through a functional pharynx taking in yolk-cells (y^2). The central cells (*b*) are much flattened to form the lining of the lumen, and the muscle-cells are lengthened radially. The two inner cells shown in dotted outline belong to the next section.

Up to the time when the embryonic pharynx becomes functional, the embryo is a solid ball of yolk in the form of a syncitium containing scattered blastomeres, with the developing pharynx at one side, in the region where segmentation began. Here and there over the surface are flattened blastomeres forming an incomplete epithelium (figs. 39–40). As the yolk is sucked in, the embryo becomes a hollow ball filled with yolk-cells (figs. 43–47). [In these and the following figures the space occupied by the yolk cells sucked in by the embryonic pharynx—the secondary yolk (y^2)—is not filled in.] Fig. 43 is a section of a nearly spherical embryo from a capsule in which some yolk still remained around the embryos. Fig. 44 is a cross-section of a flattened embryo of full size, all the yolk outside of the embryos having disappeared. In these sections the blastomeres (*b*) are scattered in the primary yolk-material (y^1) of the embryonic surface layer of the embryo, and still possess the characteristics of the earlier blastomeres, deeply-staining cytoplasm and large nucleus containing a conspicuous nucleolus. Figs. 45 and 46 show parts of sections from somewhat older embryos, where the embryonic pharynx (p^1) is degenerating and the blastomeres have multiplied so as to nearly fill the embryonic layer, very little yolk remaining among them. The embryonic pharynx disappears completely before the adult pharynx begins to form, but its relation to that pharynx appears to be the same as in *P. maculata*, as recently described by Curtis ('02). In fig. 45 the ventral side of the embryonic layer is easily distinguishable from the dorsal side by its greater thickness, and the degenerating pharynx (p^1) is on the dorsal side, as in Curtis's fig. 51, Pl. 17. The embryonic pharynx disappears so early, when many of the embryos are quite irregular in form, that it is impossible to tell whether it has a fixed position relative to the permanent pharynx or not, but my impression is that its position is variable. There is no evidence whatever that the embryonic pharynx serves as a tube leading to the anlage of the permanent pharynx, as described by Metschnikoff ('83) for *Planaria polychroa*.

Fig. 47 is from a 4-day embryo in which the pharynx-chamber appears as a split in the thickened ventral region of the embryo. In this stage pigment and rhabdites have begun to appear in the surface epithelium-cells, and rhabdite-cells are found among the embryonic cells, which are no longer like the early blastomeres, but closely resemble the embryonic cells of newly regenerated regions of adult planarians. Figs. 48 and 49 are sections of an older embryo (5–6 days), showing the permanent ectoderm well developed and full of pigment and rhabdite-cells. So far as I am able to determine, the ectoderm is formed from the outer embryonic cells and not by division of the earlier scattered epithelium-cells. There is considerable evidence that rhabdite-cells migrate from the interior to the surface and become a part of the ectoderm. In this embryo (figs. 48–51) the pharynx (p^2) is quite large and has a lumen connected with the central yolk-area (fig. 50). The yolk-area is being gradually divided up by strands of cells extending inward from the surface layer of embryonic cells to form the boundaries of the axial gut and its principal branches. Fig. 52 is from an older embryo (7 or 8 days), in which the development of the digestive tract is quite far advanced. In fig. 49 there is a section of a very young eye (*e*), the pigmented cup consisting of only 5 or 6 cells. No brain is yet distinguishable, but the lateral nerve-cords are represented by a few strands of nerve-fibers (*n*). In fig. 52 the eye is much further advanced and the nerve-cords are larger. The eyes in all embryos of this age are situated much deeper in the tissue than in the adult. There is as yet no definite endoderm, but here and there are cells with nuclei like those of adult endoderm-cells, and processes extend out from them among the yolk-cells as seen in fig. 51, *e*.

Fig. 53 is a cross-section of an embryo just before hatching (12th day). The lumen of the digestive tract is still full of yolk-cells and the endoderm-cells also contain masses of yolk (y^2). Figs. 55 and 56, endoderm-cells containing large masses of yolk, were taken from the same embryo as Fig. 53. Fig. 57 shows a similar endoderm-cell from a young planarian one day old. This cell contains one of the large vacuoles (*v*) characteristic of adult endoderm-cells, and the yolk is much disintegrated.

Thus it is perfectly plain, in this form at least, that the yolk-cells do not serve as a "vicarious endoderm" (Metschnikoff); but endoderm-cells, developed from the embryonic cells of the one germ layer, consume the yolk-cells in the same manner as they do other food material later on.

Fig. 54 is a section from the head region of the same embryo as fig. 53, showing brain (*b*) and eyes (*e*). By the fourth day after birth the yolk has all disappeared from the lumen of the gut, but masses of it are still to be seen in the endoderm-cells. The late embryos and young planarians contain a very large proportion of embryonic cells and few muscle- and gland-cells compared with mature animals. The tissue of the whole body resembles that of recently regenerated parts of adult planarians. It is interesting to note that the interval between egg-laying and the development of the permanent pharynx, eyes and nervous system in the embryo is about the same as between merotomy and regeneration of the same organs in pieces of adult planarians.

The reproductive organs develop late, and as yet have been studied in only two specimens. In one young planarian, 8 weeks old, one ovary was found, but no other reproductive or genital organs. In another, 10 weeks old, there was a small antrum with the penis just forming, but no genital pore; one ovary and three testes were found. The oöcytes in these young ovaries were small and only just distinguishable from the cells of the yoke glands, which were quite well developed. In the testes there were already mature spermatozoa.

SUMMARY.

The points that seem to need especial emphasis are:

1. In *Planaria simplissima* division of the chromosomes in both maturation-divisions is longitudinl.

2. The number of chromosomes in the maturation-divisions of the germ-cells varies frrom 3 to 6, but is usually 3.

3. In the embryological development there is nothing corresponding to the typical blastula and gastrula. After several segmentation divisions the blastomeres form an irregular group embedded in a syncitial yolk-mass which forms a part of the embryo. Some of the blasto-

meres form the embryonic pharynx; others wander through the syncitium.

4. The embryonic layer which covers the secondary yolk (y^2) taken in by the embryonic pharynx, in no way corresponds to the ordinary gastrula-stage. The solid embryo has, by sucking in yolk through its pharynx, become a hollow ball filled with secondary yoke-cells (y^2). The embryo now consists of a single layer of syncitial yolk-material, containing scattered blastomeres which feed on the primary yolk-material and multiply until they occupy the whole space previously filled by the primary yolk (figs. 43–46). Then the inner embryonic cells begin to serve as endoderm-cells to absorb the secondary yolk.

5. The axial gut and its principal branches are formed by ingrowths from the embryonic layer, dividing up the central space which is filled with secondary yolk (y^2).

6. Ectoderm, endoderm, permanent pharynx, eyes, nervous system, reproductive organs, gland- and muscle-cells are all formed by direct differentiation of the embryonic cells of the *one* embryonic or germ-layer. There is no formation of two or three distinct germ layers, nor are any of the organs formed by folding as in most other forms.

LITERATURE.

CURTIS, W. C. '00. On the Reproductive System of Planaria simplissima, a new species. *Zool. Jahrb.*, Bd. 13, 1900.

—— '02. The life history, the normal fission, and the reproductive organs of Planaria maculata. *Proc. of Boston Soc. of Nat. Hist.*, Vol. 30, 1902.

HALLEZ, P. '79. Contributions à l'histoire naturelle des Turbellariés. *Trav. de l'institut zool. de Lille*, Fasc. 2, 1879.

IJIMA, I. '84. Untersuchungen über den Bau und die Entwicklungsgeschichte der süsswasser-Dendrocœlen (Tricladen). *Zeit. f. wiss. Zool.*, Bd. 40, 1884.

KENNEL, J. VON. '79. Die in Deutschland gefundenen Land-planarien, u. s. w. *Arb. Zool. Inst. Würzburg*, Bd. 5, 1879.

METSCHNIKOFF, E. '83. Die Embryologie von Planaria polychroa. *Zeit. f. wiss. Zool.*, Bd. 38, 1883.

SCHMIDT, O. '59. Die dendrocœlen Strudelwürmer aus der Umgebung von Graz. *Zeit. f. wiss. Zool.*, Bd. X, 1859.

—— '61. Untersuchungen über Turbellarien von Corfu u. Cephalon. *Ibid.*, Bd. XI, 1861.

—— '61. Uber Planaria torva, auct. *Ibid.*

STEVENS, N. M. '01. Notes on regeneration in Planaria lugubris. *Arch. f. Entwickelungsmech.*, Bd. VIII, 1901.

DESCRIPTION OF PLATES XIII, XIV, XV AND XVI.

PLATE XIII, Fig. 1.—Ovary showing large oöcytes and oviduct full of spermatozoa (*od*). Bausch and Lamb, obj. ½ in., oc. C, camera.

Fig. 2. *a* and *b*.—Ovarian egg showing first maturation-spindle with 3 chromosomes. $y =$ yolk granule. B. and L. ⅛–C.

Fig. 3, *a* and *b*.—Same as above with 6 daughter chromosomes.

Fig. 4.—Same with 4 chromosomes (a part of one chromosome in next section).

Fig. 5, *a* and *b*.—Egg from section of a capsule before laying, showing second maturation-spindle and 3 chromosomes. *s* = sperm.

Fig. 6.—Same as above, showing first polar body and 3 chromosomes.

Fig. 7.—Somatic cell from a regenerating piece of *Planaria lugubris*, showing 6 chromosomes. B. and L. $\frac{1}{12}$–C.

Fig. 8. Egg from a capsule before laying, stained with Schneider's aceto-carmine. p^1 = 1st polar body. p^2 = chromosomes of 2d polar body. *s* = sperm. *e* = egg-chromosomes. B. and L. $\frac{1}{6}$–C.

Fig. 9.—Similar egg with 6 egg-chromosomes (*e*) and 6 chromosomes for the 2d polar body (p^2).

Fig. 10.—Egg from same capsule as 9, showing longitudinal division at *a* and *b*.

Fig. 11.—Similar egg showing 2d polar body (p^2), sperm (*s*) and egg-chromosomes (*e*).

Fig. 12.—Egg from capsule just laid showing pronuclei.

Fig. 13.—Egg from capsule 3–4 hours after laying, showing pronuclei fused but nucleoli distinct.

Fig. 14*a*.—Egg from a capsule 8–10 hours after laying, showing first segmentation-spindle with 6 chromosomes at each pole.

Fig. 14*b*.—Optical cross-section of polar plate, showing two cross-sections of each of the 6 chromosomes.

Figs. 33–35.—2-celled, 4-celled and 8-celled stages from sections of capsules, showing the peculiar relative positions of the blastomeres. Fig. 35 is a reconstruction from five sections. B. and L. $\frac{1}{6}$–C.

Fig. 36.—Section of a 32(?)-celled stage, yolk-cells breaking down at *x*, *x*. B. and L. $\frac{1}{6}$–C.

PLATE XIV, Fig. 15.—One-half of a cross-section of an unusually large testis. *a* = dividing spermatogonium. *b* = small spermatagonium after division. *c* = resting spermatgonium. *d* = resting spermatocyte of the first order. *e* = first maturation division. *f* = second maturation division. *g* = young spermatids. *h*, *i*, *k* = spermatids in later stages. *l* = spermatid apparently twice the usual size. *m* = spermatozoa. *n* = empty spermatid cells. B. and L. $\frac{1}{6}$–C.

Figs. 16–21.—Various phases of first maturation divisions, showing 3 and 4 chromosomes. B. and L. $\frac{1}{12}$–C.

Figs. 22 and 23.—Second maturation division.

Figs. 24–29.—Spermatids in various stages.

Figs. 30–31.—Spermatozoa from the testis.

Fig. 32.—Spermatozoon from the oviduct near the ovary. B. and L. $\frac{1}{12}$–C.

PLATE XV, Fig. 37.—Section of a slightly later stage (64-celled)(?), showing an irregular mass of blastomeres (*b*), a definite embryonic yolk-area (y^1) and a region of disintegrating yolk-cells (*a*). B. and L. $\frac{1}{6}$–C.

Fig. 38.—Section of a still later stage, showing larger embryonic area containing yolk-nuclei (y^1), yolk-cells (y^2), wandering blastomeres (*b*), and the beginning of the embryonic pharynx (p^1). Same magnif.

Fig. 39.—Cross-section through the central cells (*b*) of an embryonic pharynx (p^1), well-developed, but not yet functional. *c* = muscle-cells. y^1 = yolk-nucleus. *bl.* = blastomeres scattered in the yolk of the embryonic area. *e* = flattened blastomere on the surface of embryo.

Fig. 40.—Longitudinal-section through an embryonic pharynx of the same age as 39. *a* = two of the 4 large surface cells bounding the lumen. *b* = two of 4 central cells. *c* = muscle-cells. *d* = two of the 4 inner lumen-cells. *e* = a blastomere partly on the surface of the embryo. y^1 = yolk-nucleus. B. and L. $\frac{1}{6}$–C.

Fig. 41.—Cross-section of the 4 inner lumen-cells of the embryo shown in Fig. 39.

Fig. 42.—Functional pharynx, lumen open and yolk-cells entering. Same magnif.

Fig. 43—Section of embryo which has sucked in a large amount of yolk (y^2) but is still surrounded by yolk. p^1 = functional pharynx closed. b = a blastomere in embryonic layer. y^1 = yolk-nucleus of embryonic layer. B. and L. 1–C.

Fig. 44.—Section of embryo from a capsule where all the yolk has been absorbed, embryo somewhat flattened. y^2 = yolk taken in by pharynx.

Fig. 45.—Part of a section of an older embryo showing blastomeres filling the embryonic layer, slightly thicker ventral side, and degenerating embryonic pharynx (p^1).

Fig. 46.—Small part of section from same embryo showing dividing blastomeres or embryonic cells, very little yolk and few yolk-nuclei (y^1) among the embryonic cells. B. and L. $\frac{1}{5}$–C.

Fig. 47.—Later embryo (4th day) showing a very early stage in the formation of the permanent pharynx (p^2). e = ectoderm-cell containing pigment and rhabdites. r = rhabdite-cell. B. and L. 1–C.

Fig. 48.—Sections from an older embryo (6th day) showing later stage of the pharynx (p^2), well-developed ectoderm, and stands of embryonic cells beginning to divide off the central yolk-region into axial gut and its branches. a = endoderm-cells beginning to take in yolk-cells. e = very young eye, optic cup of about 5 pigmented cells. n = first appearance of nerve-cord.

Plate XVI, Fig. 49 —The same as fig. 48.

Fig. 50.—Another section of pharynx (p^2) from same embryo.

Fig. 51.—Section from same embryo, showing endoderm-cells (e) sending out processes among the yolk-cells (y^2). B. and L. $\frac{1}{5}$–C.

Fig. 52.—Section from an older embryo (7–8 days) showing older eye (e), larger nerve-cord (n) and formation of gut more advanced.

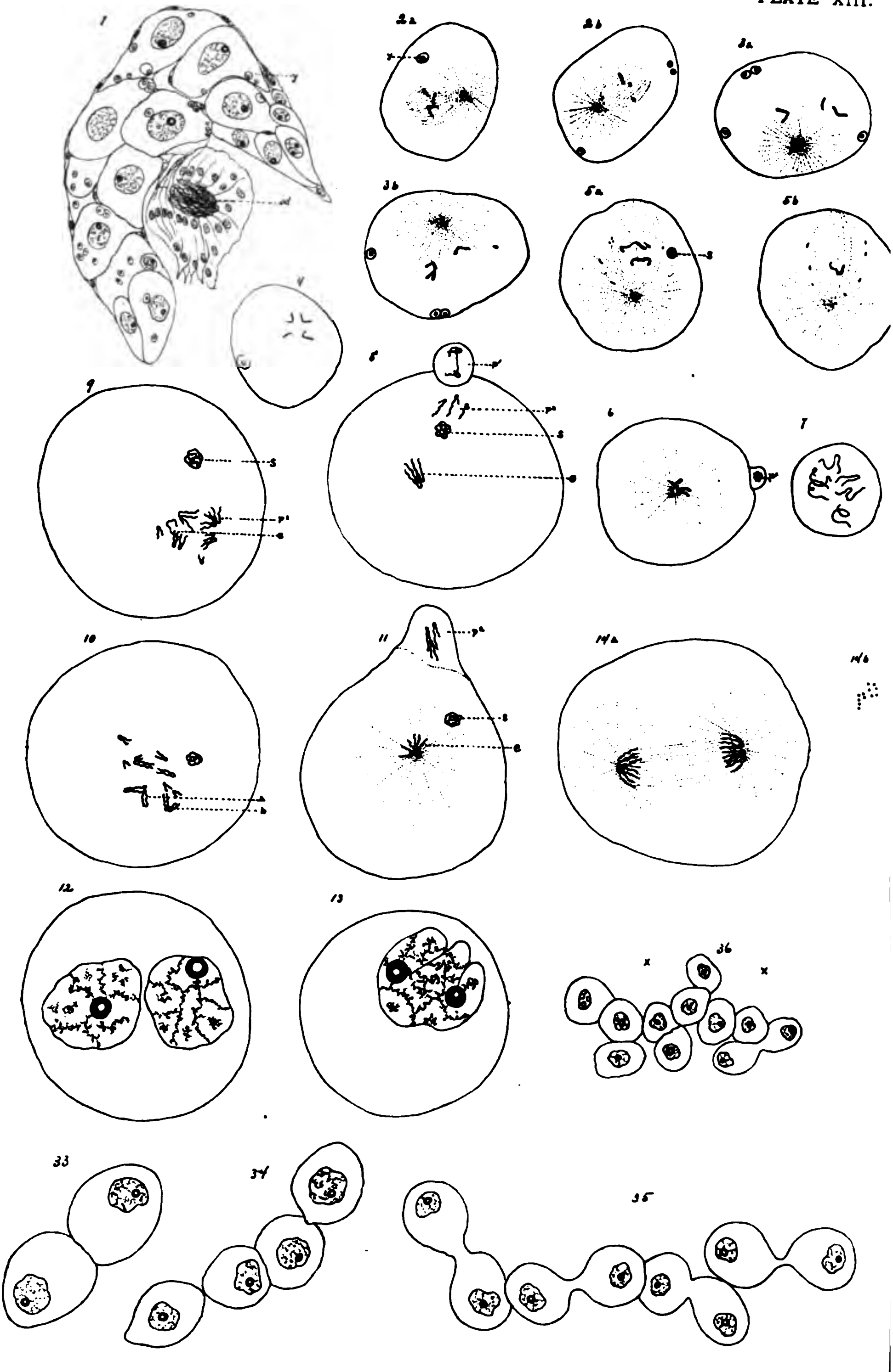
Fig. 53.—Cross-section of 12th day embryo ready to hatch, showing well-developed pharynx (p^2) and gut still filled with yolk-cells (y^2). B. and L. 1–C.

Fig. 54.—Another section from same embryo showing well-developed eyes and brain (b).

Figs. 55–56.—Endoderm-cells containing large masses of yolk (y^2), from same embryo as Fig. 53. B. and L. $\frac{1}{12}$–C.

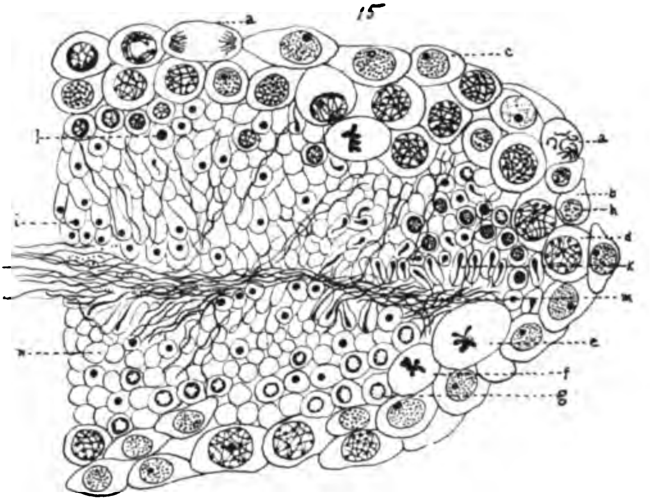
Fig. 57.—Similar endoderm-cell from a young planarian one day old, yolk considerably disintegrated.

Fig. 58.—Longitudinal section of young planarian, one day old, tail-region shorter than in adult. (e = eye. b = brain. g = gut. p^2 = pharynx B. and L. 1–a.

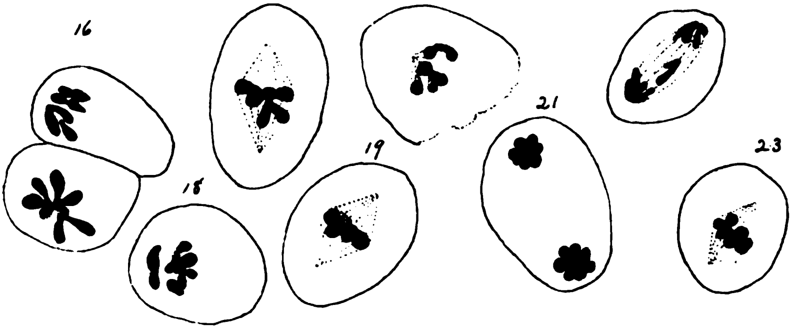

1
y
d
2a
x
2b
3a
3b
5a
s
5b
4
8
p²
p¹
s
e
9
s
p¹
e
6
p²
7
10
a
b
11
p²
s
e
14a
14b
12
13
36
x
x
33
34
35

STEVENS.

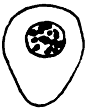
15



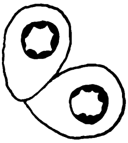
16 17 18 19 20 21 22 23



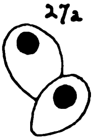
25



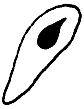
24



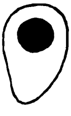
27a



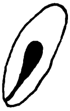
27b



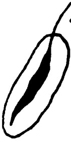
26



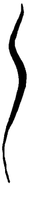
28



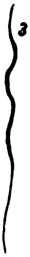
29



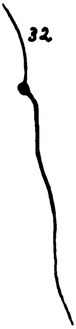
30



31

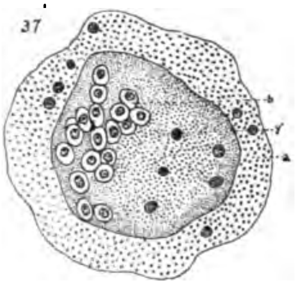


32

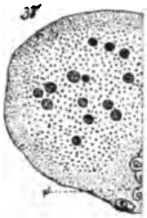


STEVENS. PLANARIA SIMPLI

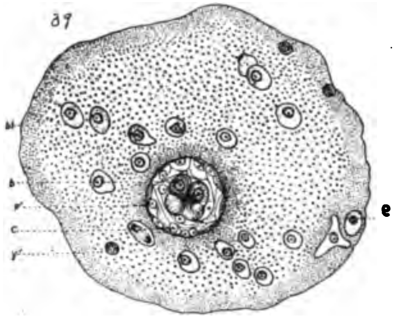
37

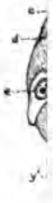


38

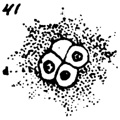


39

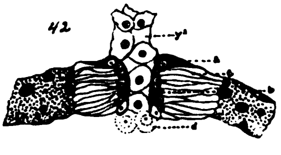




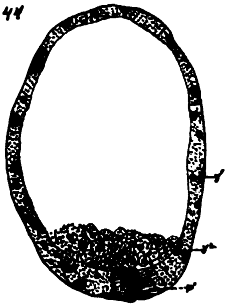
41



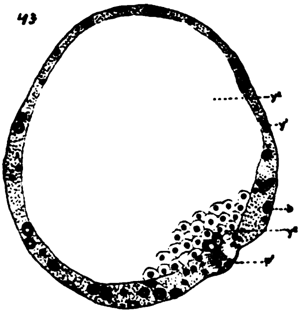
42



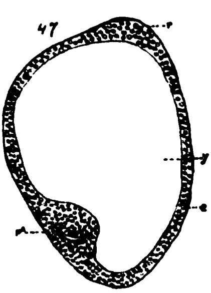
44



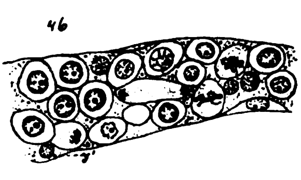
43

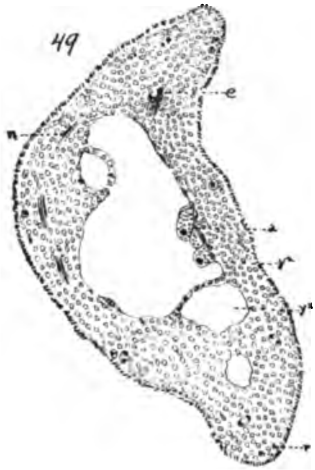


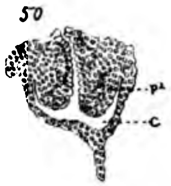
47



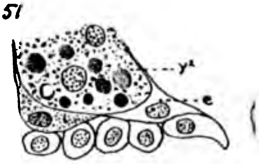
46

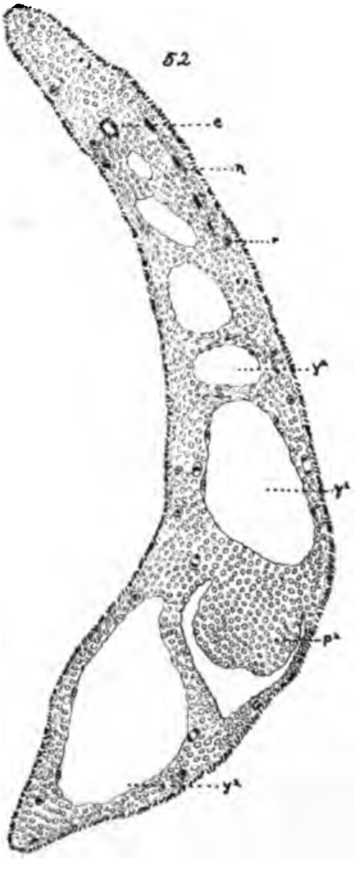


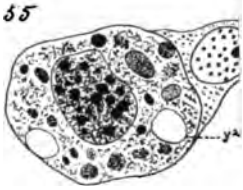

49
e
n
y²
y²
y²
r

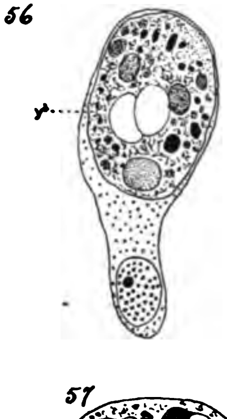

50
p²
c

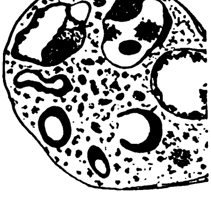
53


51
y²
e


52
e
n
r
y²
y²
p²
y²


55
y²


56
y²


57

SELF-FERTILIZATION INDUCED BY ARTIFICIAL MEANS.

BY

T. H. MORGAN.

It has long been known that the pollen of some plants will not fertilize the ovules of the same plant. The cause of this impotence has not yet been detected.

It is also known that pollen from another plant is often prepotent in those cases where normal self-fertilization may occur. It has further been shown, especially by Darwin, that the offspring from self-fertilized ovules are in general not so vigorous as those from cross-fertilized ones.

There are here two problems, which, even if they should prove to be fundamentally related, can be most profitably examined separately;— first, the problem of the inability of the male element to fertilize the female germ-cells of the same individual; and, second, the effect of self-fertilization (in those cases in which it occurs) on the offspring. Both problems appear to be within the range of experimental examination.

There are only a very few cases known amongst animals where conditions similar to those in plants have been found to prevail, although very few hermaphroditic animals appear to have been examined in this respect. Close inbreeding, which is commonly supposed to bring about deterioration in some cases, is perhaps not very dissimilar to self-fertilization. Whether in the case of inbreeding there is ultimately a loss of power to fertilize the egg, or whether the egg fails to develop after it has been fertilized, has not, so far as I know, been determined.

Castle discovered in the ascidian, *Ciona intestinalis,* a case apparently similar to those in plants. The eggs are generally incapable of self-fertilization, yet can be readily cross-fertilized; *i.e.,* the spermatozoa of an individual will not fertilize the eggs of that individual, but have the power to fertilize the eggs of any other individual.

My object in undertaking a study of this problem was, in the first place, to determine if possible the nature of the conditions that prevent or interfere with self-fertilization; and in the second place, I was not without hope of being able to find some way in which self-fertilization could be artificially induced. As will appear in the sequel, these two questions are not two sides of the same problem; for, while it has been possible to discover the means of bringing about self-fertilization, it still remains to be definitely determined what conditions in the egg normally prevent the entrance of the spermatozoa of the same individual.

Since Castle's observations had shown that the ascidians offer favorable material for a study of this sort, I first turned my attention to this group, using the three most available species found at Woods Hole, or in the vicinity; namely, *Ciona intestinalis, Molgula manhattensis,* and *Cynthia partita* (*Styela* sp.). The work was done while holding the Bryn Mawr Table at the Marine Biological Laboratory, from June to September, 1903. Owing to the scarcity of Ciona I have not been able to work out completely a number of important problems connected with one of the two main questions that I examined. In the near future I shall hope to complete this side of the investigation.

EXPERIMENTS WITH CIONA INTESTINALIS.

The ovary of Ciona is a sac-shaped body of fair size lying loosely attached in the coil of the intestine. It can easily be removed without cutting into the testis. Its lumen contains some of the ripe eggs, but the majority of these are in the oviduct. The oviduct can readily be opened and the eggs set free without cutting into the vas deferens, which follows a course parallel to the oviduct. If the animal is kept isolated for 24 hours the oviduct becomes greatly distended with eggs, and after another 24 hours even more eggs may have accumulated. The eggs are laid normally in the early morning, at dawn, and Castle has recorded that Ciona deposits its eggs and sperm with the regularity of the rising sun. The rough handling incidental to removal and isolation appears to cause Ciona to retain its eggs for several days. The individuals to be used were isolated, as a rule, from 24 to 48 hours, and in most cases were rinsed in fresh water before opening. It was not found necessary to boil the water; for check experiments showed that eggs left to themselves were never fertilized by stray spermatozoa in the sea-water. Since Ciona deposits its eggs only in the very early morning, the chances are very slight that functionally active spermatozoa would be present in the sea-water in the late morning and in the afternoon when the experiments were carried out.

The eggs of Ciona are surrounded by a rather thick membrane. Standing out like broad spikes over the surface of the membrane, and forming a beautiful aureole around the egg, are the transparent follicle cells, each with a shining drop in its outer end.

A number of preliminary experiments confirmed Castle's conclusion that self-fertilization is rarely possible in Ciona intestinalis. The evidence, however, on which Castle based this conclusion is not altogether satisfactory, since he records many cases in which self-fertilization occurred. Instances are cited in which isolated individuals gave 90, 25, 16, 5, 4, 0 per cent. of self-fertilized eggs. Castle supposes that, in the first of these cases at least, the spermatozoa of one day fertilized the eggs of the next, but it has not been shown that the spermatozoa have this power if left so long in sea-water. The same individuals that had been used for these isolation experiments were killed (after being washed in 90 per cent. alcohol), and the eggs and sperm of each taken out and mixed together. The results gave 50, 4, 1, 1/3, 0 per cent. of self-fertilized eggs. The same experiment repeated with fresh individuals gave 50, 12½, 10, 5, 2, 0 per cent. of self-fertilized eggs. From these figures it is clear that in some cases a considerable amount of self-fertilization occurred, unless there was some source of error in the experiment. In fact, Castle believes that in those cases where a large number of eggs were fertilized there was some contamination. My own results with Ciona have never given so large a percentage of self-fertilized eggs, and I am inclined to attribute this result in part to the precaution that I took to isolate the individuals the day before they were to be used. I have rarely seen more than from 1 to 10 per cent. of self-fertilized eggs segment, and in the greater number of cases not a single egg segmented. On the other hand I found, as did Castle, that as a rule 100 per cent. of cross-fertilized eggs develop, to which statement I should add, provided the spermatozoa are in "good" condition.

What is the meaning of these remarkable facts? Why do not the sperm fertilize the eggs produced by the same individual, and yet fertilize those of any other individual? A number of possibilities readily suggest themselves, and since the following pages record an attempt to test these suggestions they may be briefly mentioned here:

1. That the spermatozoa are not made sufficiently active by secretions from the eggs of the same individual, but by those from the eggs of any other individual.
2. That the spermatozoa are not "attracted" to the eggs of the same individual.
3. That the egg contains, or secretes some substance that lessens the activity of the spermatozoa of the same individual.
4. That some mechanical difficulty prevents the spermatozoön from entering the egg of the same individual.
5. That even if the spermatozoön enters, it can not fertilize the egg of the same individual, in the sense of causing the egg to begin to develop.

In order to discover if the lack of power to self-fertilize the eggs is due to the absence of some substance around the eggs that excites the spermatozoa, the following experiment was carried out. The eggs of an individual (A) were taken from the oviduct. Similarly the eggs of another individual (B) were also taken out. Then the ovary of (A) and that of (B) were crushed separately, and a little sea-water was added. The eggs of (A) were then allowed to soak in the crushed ovary extract of (B) and those of (B) in the extract of (A). After a short time the sperm of (A) with a little water was added to the (A)-eggs, and the sperm of (B) to the (B)-eggs. If the sojourn of the eggs in the extract of the ovary of another individual has the postulated effect, or if the presence of the extract of the ovary of another individual has the postulated effect on the sperm, fertilization ought to have occurred. The results showed, however, that fertilization did not take place.

This experiment was performed four times, giving eight sets in all. In six of these sets not a single egg segmented. In two others a very few eggs segmented (6 per cent. in one, 5 per cent. in the other), but this sometimes occurs in self-fertilized eggs not treated in any special way. Moreover there may have been contamination in the latter case.

Another experiment similar in some respects to the last was also carried out. The heart of one individual was opened and the blood collected. The eggs of another individual were put into this blood and allowed to stand. Later, sperm of the same individual was added in sea-water, but no fertilization occurred in one set and only one per cent. in the other. Check eggs were also kept in this experiment to make certain that no sperm had accidentally gotten into the blood. That none were present was shown by the fact that no fertilization took place. It is evident from this experiment that self-fertilization can not be brought about by soaking the eggs in the extract from the ovary or in the blood of another individual, although the somewhat high percentage of self-fertilized eggs that segmented in two cases after treatment with the ovarian extract may have resulted from the influence of the extract on the spermatozoa.

If the spermatozoa are excited to greater activity by the presence of the eggs of another individual it seemed not improbable that this might be directly observed. Therefore, I placed some of the sperm with the eggs of another individual and more of the same sperm with the eggs of the same individual, and compared the two preparations under the microscope. The spermatozoa of Ciona are not very active as a rule, nor do they accumulate in crowds around the eggs, as they do in many other animals, or at least not to any marked extent. It seemed to me in both cases that sometimes the spermatozoa were more active immediately in the vicinity of the eggs, and in the spaces between the follicle cells, but as they also show the same activity around pieces of the tissue of the body of the same or of another individual I have not laid much stress on this observation, or accredited the results to the presence of an exciting substance. At times I have thought that the spermatozoa were more active around the eggs of another individual than around the eggs of the same individual, but as there is no very accurate means of determining their relative motility, unless very marked, I should not wish, as yet, to give a final answer to this question. It is certain that there is no such great difference in the behaviour of the spermatozoa in the presence of the eggs of the same and of another individual as to suggest that the difference in the result is connected with this factor. And even if this were the case, the influence probably extends for only a short distance from the surface of the egg, as the following experiment shows.

The eggs were taken from the oviduct, great care being taken not to injure the sperm-duct. The eggs from another individual were collected in the same way. An equal number of eggs from each were put together and fertilized with the sperm from one of the individuals. In another dish another lot of the same eggs were mixed half and half, and these fertilized with the sperm from the other individual. In each of these two sets half at least of the eggs should be fertilized by the other sperm, but half should not be fertilized unless the eggs of one individual exert some influence that causes the sperm to fertilize the eggs of the same individual also. It was found that only about half of the eggs were fertilized. This result shows that the fertilization i probably not due to some substance set free by the eggs that acts on the sperm or at least that if such a substance is set free its action is confined to the immediate vicinity of the egg. The experiment does not show, however, whether the egg, or its membranes, may not contain some substance that prevents the spermatozoa from entering the eggs of the same individual. Even if such a substance is set free from the eggs it may not have had time in my experiment to accumulate sufficiently in the surrounding water to have prevented the spermatozoa from fertilizing the other eggs, which may be quickly entered. This view can be tested by letting eggs stand in a small amount of water for a long time, then taking out some sperm from the same individual, first making it active by placing it in sea-water, and then putting it into the water in which the eggs have stood. On the hypothesis these sperm should soon be brought to rest, and if then the eggs of another individual are added, they should not be fertilized, or at least not in the same proportion as when the sperm is taken directly from the oviducts, put into sea-water, and then added to the eggs.

THE INFLUENCE OF ETHER ON CROSS-FERTILIZATION.

EXPERIMENTS WITH CIONA.

My first experiments with ether were made in order to determine whether when eggs are etherized it might not be possible to self-fertilize them. The results turned out somewhat differently from what I had anticipated, for although I found that it was possible to self-fertilize the eggs in ether-solutions, the result seemed to be due to the action of the ether on the sperm rather than on the eggs.

The experiment was first made with Cynthia, which in most cases has very sluggish spermatozoa. I observed that the first effect of the ether was to make the sluggish sperm very active, and even greatly quickened the activity of already active sperm. Furthermore I found that spermatozoa that scarcely moved at all in sea-water became active in the ether-solutions. Finally I found that in ether-solutions of certain strengths the eggs of Cynthia and of Ciona could be self-fertilized. The eggs behave in this respect so capriciously that I was obliged to carry out a large number of experiments in order to determine the conditions that lead to the self-fertilization of eggs in ether-solutions. The outcome was only partially satisfactory, but the experiments opened up a field for research, in which it may be possible to obtain further results of interest.

The experiments with ether were carried out as follows: At first I used a nearly saturated solution of ether and diluted it a half, or a fourth, etc. In the later experiments I used solutions of known strength. It was found by trial that the solutions were effective between 0.25 and 5 per cent. Some of the results may now be given in detail.

Experiment I. The eggs were removed from an individual that had been isolated 20 hours. The sperm was also taken out, and, together with the eggs, was put into ether-solutions, 5, 2, 1, 0.7, 0.5 per cent. in sea-water. After 5 minutes, and again after 10 minutes, the eggs were removed to pure sea-water. The eggs were injured by the ether in the strongest solution, but nevertheless one segmented. In all of the other solutions about 80 per cent. of the eggs divided; the most in the weaker solutions.

Experiment II. In this experiment the eggs and the sperm were put together into ether solutions of 5, 2, 1, 0.7, 0.5 per cent. Some of the eggs were transferred to water after 5 and 10 minutes, but others were left in the solutions. In the strongest solution the eggs were killed. In the others the following results were obtained:

Ether	Eggs segmented 5 minutes in ether.	Eggs segmented. 10 minutes in ether.
2. per cent.	20 per cent.	25 per cent.
1. "	5 "	75 "
0.7 "	2 "	2 "
0.5 "	10 "	5 "

It is clear that the stronger solutions gave the best results, and that ten minutes immersion was better than five minutes. None of the eggs that were left in the ether-solutions segmented. This does not mean that they were not fertilized, but that the ether so injured the eggs after a long immersion, that they failed to develop. Several check experiments were also made in this case. In one the eggs were not self-fertilized but were put into a 5 per cent. ether-solution, and transferred after ten minutes to sea-water. They did not segment, nor did a few that were left behind in the ether solution. In another check series the eggs were not fertilized, and were left in sea-water. None segmented, which shows clearly that the ether in the preceding experiment was in some way responsible for the self-fertilization of the eggs. It should also be recorded that tadpoles developed from all the fertilized eggs that had been in the ether-solutions.

Experiment III. The eggs and sperm of the same individual were put into ether-solutions of 3, 2, 1, 0.7, 0.5 per cent., and were removed to sea-water after 10, 20 and 30 minutes.

Ether	10 min.	20 min.	30 min.
3. per cent.	20 per cent.	20 per cent.	0 per cent.
2. "	33 "	17 "	90 "
1. "	50 "	25 "	10 "
0.7 "	50 "	20 "	0 "
0.5 "	20 "	20 "	12 "

The table shows that eggs segmented in all of the solutions, best however in the stronger solutions, although in one case the eggs became so injured by the ether that they did not develop further than the segmentation stages. In a check series, in which the self-fertilized eggs were put into sea-water, about ten per cent. of the eggs segmented. There may have been some source of contamination, or else, and this seems more likely since the individual had been isolated 20 hours, self-fertilization took place on a larger scale than usual.

Experiment IV. Eggs and sperm were mixed in ether-solutions of 4, 2, 1, 0.5 per cent.

Ether	2 min.	4 min.
4.	0	0
2.	40	1
1.	35	30
0.5	5	1

These results show that the 4 per cent. solution was too strong, while the 0.5 per cent. solution appears to have been too weak. The injurious action of the 4 per cent. solution appears to have been mainly on the sperm rather than on the eggs, for these eggs after they had been in sea-water 4 hours were capable of being cross-fertilized, and 25 per cent. of them developed.

Experiment V. Eggs and sperm were put into a 2 and into a 0.5 per cent. ether-solution and removed after 5 minutes.

Ether	5 min.
2.	90
0.5	90

It is interesting to note in this case, in which so large a percentage of the eggs were self-fertilized in ether, that of several hundred eggs of the same individual, to which sperm was added, but which were kept in sea-water, not one segmented. It was also found, and will be referred to again later, that when the sperm alone was put into a 2 per cent. soluton of ether for five minutes, and was then added to eggs of the same individual in sea-water, 70 per cent. of the eggs segmented.

Experiment VI. In this experiment the eggs and the sperm were put into ether-solutions of 2 and of 0.5 per cent. and removed after ten minutes. In one lot the eggs were self-fertilized, in the other they were cross-fertilized.

	Self-fert.	Cross-fert.
Ether	10 min.	10 min.
2.	0	100
0.5	0	100

In this case although no self-fertilization took place, all the crossed eggs which had also been in the ether-solution developed, showing that the solutions have no baneful effect on cross-fertilization. The lack of self-fertilization shows that the sperm were not sufficiently acted upon by the ether-solutions employed to effect self-fertilization.

Experiment VII. This experiment shows how slight a difference in the conditions may cause great differences in the result. The individuals had been isolated 48 hours. One lot of self-fertilized eggs was kept in water and allowed to stand there 20 minutes. The eggs with the surrounding sperm were then put into ether-solutions of 2, 1 and 0.5 per cent. for 20 minutes, and then returned to sea-water. None of these segmented. Another lot of eggs from this individual were mixed with sperm of the same individual and put into a 2 per cent. ether-solution for ten minutes and then carried back to sea-water. Here 95 per cent. of the eggs segmented. On the other hand some of these same eggs taken from the ether after 5 minutes did not divide. The following experiments were also carried out with other self-fertilized eggs of the same individual.

Ether	15 min.	30 min.
2.	45	0 (only 3 eggs)
1.	30	40

Experiment VIII. The eggs and the sperm of one individual were mixed in ether-solutions of 4, 3, 2, 1, 0.7, 0.5 per cent., and removed after 10, 20, 30, 60 minutes to sea-water. It was noticed that the spermatozoa were very sluggish in sea-water, and although somewhat more active in the ether solutions, yet their activity was not marked. Of the eggs, which appeared to be in excellent condition, only three segmented, two in the 1 per cent. (20 minutes) and one in the 0.5 per cent. (20 minutes.)

The eggs that had not segmented after the ether treatment were fertilized, after they had stood 6 hours, with sperm from another individual, and three quarters of an hour later nearly all had divided normally into two cells. It was observed that the spermatozoa of this second individual, used for cross-fertilization, were also very inactive in their own fluid. They seemed to be more active in the extract from the ovary of the first individual. This ovary had also stood 6 hours in sea-water.

At the same time another experiment was made in which the eggs of another individual, that had been isolated for 24 hours, were put into ether solutions of 3, 2, 1 per cent. for 10, 20, and 60 minutes, and then returned to sea-water. None of these eggs divided, except one in the 3 per cent. solution (60 minutes). It was observed in this case that the sperm was inactive even in the ether-solutions, but nevertheless this same sperm, not in ether, cross-fertilized the eggs of the other individual in the preceding series, and also, as stated, appeared to be somewhat active in the extract of the ovary of the other individual.

Experiment IX. The eggs and sperm together were put into ether-solutions of 5, 4, 0.7 per cent. The sperm were active even in sea-water. The individual had been isolated for two days.

Ether.	10 min.	20 min.	30 min.
5.	0	12	0
4.	10	5	(7 out of 8 eggs)
0.7	12	10	(9 out of 10 eggs)

There was another series in this set in which the ether was stronger (about one-half saturated). None of the eggs from this solution segmented, but they became filled with clear spots. In another check series, self-fertilized but kept in sea-water, none of the eggs developed.

Experiment X. Sperm alone was put into ether solutions of 6, 4, 1, 0.5 per cent. It was removed (along with some of the surrounding fluid) and added to the eggs after 2 and 10 minutes.

Ether	2 min.	10 min.
6.	0	0
4.	0	0
1.	20	10
0.5	4	50

It appears from this experiment that it suffices to put only the sperm into the ether-solutions to bring about self-fertilization, but it should not be overlooked that a certain amount of the ether is carried over with the sperm when the latter is added to the eggs. The amount, it is true, will be small, since the eggs stand in water which further dilutes the ether, but so long as this source of error is present, and it is very difficult to remove it entirely, the result does not show conclusively that the ether acts on the sperm alone, although I think this is the more probable interpretation.

A check series of experiments was also made in which both eggs and sperm were put into solutions of the same strength as those given above, for 15 minutes and then removed to water.

Ether.	15 min.
6.	0
4.	0
1.	100 (but only ten eggs present.)
0.5	90

It is evident from both of the foregoing tables that only the weak solutions were effective, and from the first table it appears that this must have been the result of injury to the sperm. It can easily be seen that the eggs also are killed in a few minutes by a 6 per cent. solution of ether.

RYN MAWR COLLEGE - MONOGRAPH

Experiment XI. Eggs from the oviduct were put into ether solutions of 1 and 0.5 per cent. for one-half and three-quarters of an hour; then washed in a small amount of fresh water and fertilized with the sperm from the vas deferens of the same individual. The experiment was carried out primarily in order to see if the eggs were affected by the solutions, so that they could be subsequently self-fertilized, but it is obvious that this test is not a good one, since the eggs will carry with them, despite the partial washing in water, some of the ether which may then act on the sperm. The results were as follows:

Ether	½ hour	¾ hour
1.0	0	0
0.5	4	5

Without a check series, which unfortunately was not made, it is difficult to decide whether the small number of eggs that were self-fertilized was due to the action of the ether on the eggs or on the sperm. The experiment must be repeated on a more elaborate scale.

Experiment XII. In this experiment with two individuals, weaker solutions of ether were used. In one lot the sperm alone was put into ether, and then added to the eggs. In the other lot both eggs and sperm were put together into the ether. I omitted recording the time in the ether, but it was probably about five minutes.

Ether	Sperm and Eggs in Ether	Sperm only in Ether
1.0	0	0
0.5	0	2
0.25	0	0

Ether	Sperm and Eggs in Ether	Sperm only in Ether
1.0	20	0
0.5	50	0
0.25	85	4

This experiment shows that the sperm of the first individual was incapable of self-fertilization, even with the ether present. In the other individual, the sperm was good, and there was a great deal of it; hence, no doubt, the excellent results in the first column. What is especially significant is that the best results were obtained when the eggs and the sperm were put at the same time into the solution together. This may mean the ether has some effect on the eggs as well as on the sperm, or that the most effective period of activity for the sperm is immediately after it comes into contact with the ether. My experiments do not suffice to settle this point, but that the spermatozoa are still capable of cross-fertilizing, after they have been in the ether for some time, is shown by the following result. After four hours the eggs of the first individual were mixed with the eggs and the sperm of the second individual. Later it was found that all the unsegmented eggs had been fertilized. The ether had no doubt largely evaporated.

The preceding twelve experiments with ether-solutions gave definite results, although in a few cases the number of eggs self-fertilized was small. It should be stated that there were ten other individuals in which self-fertilization in ether did not take place. This does not detract, I think, from the value of the successful experiments, because, as has been shown, the sperm is sometimes incapable of fertilizing even the eggs of another individual. The following experiments were carried out in order to examine this question further. It will be observed that parallel experiments with ether were also performed.

In each series five individuals were used. The eggs of each were fertilized with the sperm of every other individual. The following scheme shows the order in which the eggs were crossed. An individual having been opened, the eggs were removed from its oviduct and distributed in five dishes, A-A. Another individual was then opened (using, of course, different scissors, pipettes, etc.) and its eggs distributed to the next line of dishes, B-B. The same method was followed for the other three individuals. The sperm, a, of the first individual was then taken out and put into a small amount of water. It was then distributed to one set of eggs from each of the other individuals, B, C, D, E; then the sperm of B was taken out and applied to another set of eggs. The process was repeated until all the eggs were supplied with sperm. The sperm in each case is indicated in the table by the small letter used as an exponent. The first set of A-eggs was as a rule fertilized with the e-sperm and the last set of E-eggs with the a-sperm.

Experiment XIII.—

E^a 100	E^b 100	E^c 100	E^d 100	E^a 85
D^a 100	D^b 100	D^c 100	D^e 100	D^e 100
C^a 98	C^b 100	C^d 75	C^d 75	C^e 100
B^a 99	B^c 100	B^c 100	B^d 100	B^e 100
A^e (omitted to add sperm)	A^b (omitted to add sperm)	A^c 100	A^d 85	A^e 99

In this experiment practically all of the eggs were fertilized by the sperm of another individual. When fewer than the total number segmented (fertilized), immature eggs may have been present. As a check series A, B, C, D and E were self-fertilized. None segmented, except in E, where two eggs out of the twenty present, *i. e.,* 10 per cent. divided.

The two ether series (self-fertilized) of these same eggs gave the following results:

Ether	A	B	C	D	E
0.5	10	1	0	80	30
1.0	50	2	0	0	0

Experiment XIV. An experiment similar to the last was carried out, with five other individuals, and gave the following results:

E^a 0	E^b 0	E^c 30	E^d 100	E^a 4
D^a 0	D^b 0	D^c 0 (one egg)	D^e (no ripe eggs)	D^e 100
C^a 0	C^b 0	C^d (no eggs)	C^d 100	C^e 100
B^a 0	B^c 0	B^c 0	B^d 50	B^e 100
A^e 0	A^b 0	A^c 0	A^d 100	A^e 100

Despite a few slight discrepancies in this table, the main result is clear. In only two of the five individuals was the sperm capable of cross-fertilization, namely, the e-sperm and the d-sperm.

There was also a self-fertilized series of these eggs, and in this not any of the eggs segmented. The ether series gave the following results:

Ether	A	B	C	D	E
0.5	0	0	0	0	10 (only ten eggs.)
1.0	0	0	?	2	50

It becomes evident from this result that the frequent failure of the sperm and eggs (mixed together) to self-fertilize in ether is due to the poor quality of the sperm. The poor sperm does not cross-fertilize, and presumably for the same cause it can not always be made to self-fertilize even in the ether. That sperm that is too poor to cross-fertilize may sometimes self-fertilize with the help of ether I hold to be possible. I regret that I did not attempt to determine whether poor sperm, that will not cross-fertilize, can be made to do so by means of ether, but other experiments lead me to think that it would often do so.

Experiment XV. In the following experiment all of the sperm appears to have been good except that of A, whose eggs, however, were in excellent condition.

E^a	2	E^b	85	E^c	90	E^d	0 (unfertilized)	E^a	0
D^a	1	D^b	100	D^c	20	D^e	90	D^e	100
C^a	70	C^b	100	C^d	100	C^d	100	C^e	90
B^a	5	B^c	100	B^c	100	B^d	100	B^e	100
A^e	0	A^b	70	A^c	100	A^d	100	A^e	100

It is clear that the a-sperm was poor, although it did well in C^a in which 70 per cent. of the eggs divided.

There was also a self-fertilized series in which none of the eggs segmented, except 5 per cent. in B. (In C, 90 per cent. of the eggs divided, but this may have been due to accidental contamination.) In the ether series the following results were obtained.

Ether	A	B	C	D	E
0.5	0	25	50	0	2
1.0	0	0	10	0	0

In this case although the spermatozoa of B, C, D, E were capable of crossing, they self-fertilized in ether very poorly, except in C, where good results followed.

Experiment XVI. In this experiment again only the first individual produced poor sperm, yet it did fairly well in one case, and in ether gave some results.

E^a 5 [illegible]	E^b 100 [illegible]	E^c 80 [illegible]	E^d 100	E^a 70
D^a 0 [illegible]	D^b 100 [illegible]	D^c 10 (few eggs)	D^e 100	D^e 30
C^a 0 [illegible]	C^b 100	C^d 100	C^d 100	C 100
B^a 25 (only 4 eggs)	B^c (no eggs)	B^c (no eggs)	B^c (no eggs)	B^e (no eggs)
A^e 75	A^b 100	A^c 100	A^d 100	A^e 100

In the self-fertilized series no eggs segmented. The ether series gave the following results:

Ether	A	B	C	D	E
0.5	2	4	2	30	0
1.0	50 (only 8 eggs)	12 (only 8 eggs)	0	10	30

The experiments recorded in Exp. XIII to XVI show that the sperm is at fault when cross-fertilization does not take place. In fact, eggs in the oviduct seem always to be capable of cross-fertilization. It is also evident that it is more difficult to get results with ether when the sperm does not cross-fertilize well, than when it does act well in this way. From this it seems to me very probable that when the ether fails to bring about self-fertilization the fault lies with the sperm. We may perhaps even go further and conclude that the action of the ether in bringing about the self-fertilization is on the sperm alone, but I am not in position to prove positively that the action of the ether on the eggs may not also enter into the result.

In concluding my account of these experiments on Ciona, I should like to point out that I had constantly in mind the possibility that the ether might produce parthenogenetic segmentation, and that the sperm had in reality nothing to do with the result. It was abundantly shown, however, that this was not the case, and in the few experiments in which I put this view to the test, by keeping eggs without sperm in ether-solutions of various strengths, I got no results when the eggs were returned to water. It should be noted in this connection that Lyon[1] has recently recorded that he was unable to cause artificial parthenogenesis in Ciona intestinalis at Naples by any of the ordinary means that excite this development in other eggs.

---

[1] American Journal of Physiology, IX, July, 1903.

I shall discuss later the view as to whether eggs may be entered by the sperm of the same individual, but fail to develop unless incited to do so by some external agent.

It has been pointed out in the preceding pages that the eggs of Ciona may be fertilized after they have been in sea-water several hours. I made a test of this again in the following experiment:

Experiment XVII. Some eggs were cross-fertilized at once, others after 30, 80, 125 minutes, with fresh sperm from the same individual. All the eggs developed. A striking fact was observed in this case. The eggs fertilized late began to segment after a shorter interval than did those fertilized at once, so that at the 32-cell stage those fertilized last were only one division behind the first set, and no doubt soon caught up. It appears that a ripening process goes on in the egg as it stands in the sea-water, so that it begins to segment more quickly after it is fertilized than does an egg fertilized as soon as removed from the oviducts. It even appeared that after the first cleavage the rhythm of division was quicker in the eggs whose fertilization had been delayed, but this point needs a special examination which I have not yet made. The discovery is all the more significant because the first polar spindle is already formed in Ciona while the egg is in the oviduct, and the spindle remains resting in the equatorial plate stage until the egg is fertilized; hence the difference in time of segmentation can not be accounted for by the time required for the breaking down of the egg-nucleus and for the formation of the polar spindle after the egg has been removed from the animal. Some change must take place in the sea-water, which, while it does not cause the polar spindle to pursue its development, yet causes the developments that take place after the spermatozoön enters to go on more rapidly.

EXPERIMENTS WITH CYNTHIA

The ovaries of Cynthia extend far forward, and have a very short oviduct. Each ovary—there appear to be two in each individual—is double, the halves being united at the distal end. Owing to the close proximity of the ovary to the surrounding tubes of the testis, it is possible only by very careful manipulation to get the eggs out of the cavity of the ovary without cutting into the testicular tubes. When it was necessary to separate the eggs from the sperm of the same individual, I have carried out this operation, but in general the ovaries and the testes were cut up together.

For the purpose of studying the effects of self-fertilization Cynthia is in many respects inferior to Ciona because self-fertilization takes place to a very large extent. On the other hand, if check experiments are used for each individual, this factor can be estimated, and the very fact that Cynthia does self-fertilize its own eggs to such an extent gives an opportunity to examine other aspects of the problem. A much more serious difficulty is met with in that artificial cross-fertilization is often unsuccessful in this species. Even when the eggs and sperm from a large number of individuals are mixed together, fertilization may not take place; but in curious contrast to this result are the following observations on the egg-laying processes of this animal kept in aquaria. On several occasions a number of individuals were put together in the same dish. About 5 o'clock in the afternoon one after another began to send out jets of eggs and of sperm producing the effect of a lively cannonading. Under these circumstances it was found that every single egg was fertilized. Perhaps only ripe individuals sent out their eggs and sperm, or perhaps the eggs were mature in all individuals, and the sperm from one or two individuals may have sufficed to fertilize all of the eggs. In general it is, I think, the sperm of Cynthia that is not good. Certainly the spermatozoa are often very sluggish when taken from the testis and put into water. May it not be possible that when the eggs are laid, Cynthia secretes some other fluid that makes the sperm active? This point needs further investigation.

The best means that I found to determine the extent to which self-fertilization of the eggs of Cynthia may take place was to isolate some of the individuals early in the day, and observe in those that emitted eggs and sperm in the late afternoon the percentage of eggs that segmented. The following four records were obtained in this way: For August 11—33, 10, 100, 95, 95, 75, 10 per cent. For August 16—30, 30, 10, 1, 75, 90, 85 per cent. For August 19—33, 0, 10, 4, 4, 0. For August 20—12, 4. A much larger number of individuals gave off neither eggs nor sperm, and some produced sperm and no eggs, and *vice versa.* The results in the above list show all conditions from perfect self-fertility to absolute self-sterility, although some of the latter cases may have been due to no sperm being given off.

A few preliminary trials were made with two (A and B), and with three (A, B, and C) individuals. The scheme of crossing is given in the following diagrams:

For Two Individuals.	
A^a	B^b
A^b	B^a

For Three Individuals.		
A^a	B^b	C^c
B^a	A^b	B^c
C^a	C^b	A^c

A few examples of the results with two individuals are as follows:

A^a 0	B^b 0	A^a 0	B^b 0
A^b few	B^a 15	A^b 10	B^a 10
A^a 0	B^b 0 (later rarely one)	A^a 0	B^b 0 (one egg)
A^b few	B^a 20	A^b few	B^a few
A^a 0	B^b 0 (one egg)		
A^b 50	B^a 0		

Comparing the self-fertilized eggs with the crossed-eggs, it is clear that while self-fertilization did not take place in nine cases, and in only one egg in the other case, yet cross-fertilization more frequently occurred, but never so completely as when many individuals normally deposited their eggs and sperm together. In addition to these cases there were three others in which none of the eggs, neither self- nor cross-fertilized, segmented. One of the results with three individuals is given in the next table:

A^a 0	B^b 2	C^c 0
B^a few	A^b very few	B^c 25
C^a 75	C^b rare	A^c 4

In this experiment the a- and c-sperm did not self-fertilize, but the former did well with C- and the latter with B-eggs. The b-sperm self-fertilized to a slight extent, but did no better with the A- and with the C-eggs.

In the next series the results are more striking:

A^a	0	B^b	0	C^c	0
B^a	95	A^b	50 (rest not ripe)	B^c	1
C^a	50	C^b	very few	A^c	50

Here none of the sperm self-fertilized the eggs. The a-sperm did quite well with the B- and C-eggs (95 and 50 per cent). The b-sperm did well with the A-eggs, but not with the C-eggs. The c-sperm did well with the A-eggs, but not with the B-eggs. It may appear from the preceding table that there is something more involved than simply the question of good sperm, for the same sperm appears to act differently with different eggs.

Another experiment with three individuals gave no eggs self-fertilized, but good cross-fertilizations with the c-sperm; less good with the b-sperm. These experiments should be carried out on a larger scale, and at different times of the year, but they suffice to show that self-fertilization is very infrequent when the process is an artificial one. It takes place to a considerable extent in some cases when eggs are normally laid. Moreover the artificially crossed eggs do not segment nearly so well in Cynthia as in Ciona.

The next experiment shows the action of ether on self- and cross-fertilized eggs. Some of the eggs and sperm of one individual, A, were removed and put into sea-water. Other eggs, A^a, were self-fertilized in an ether-solution, and a third lot, A^b, were crossed with sperm from B (A-sperm was also present). The same process was carried out with B which was crossed with sperm from A.

A	0	B	0
A^a	few	B^b	few
A^b	very few	B^a	very few

The results show that the self-fertilized eggs in ether did as well as those that were crossed, but none of the eggs in water alone, with their own sperm, segmented. Another similar experiment with two other individuals gave the following results:

A	0	B	0
A^a	0	B^b	0
A^b	50	B^a	(only one egg 0)

In another set the ether appears to have been too strong, yet 50 per cent. of A^b divided.

In another experiment, 10 per cent. of the self-fertilized eggs in ether segmented, and 50 per cent. of the crossed.

In another, 5 per cent. of the self-fertilized eggs in ether segmented, and 75 of the crossed.

The next set is more instructive:

A	0	B	0
A^a	100	B^b	0
A^b	2	B^a	4

It is clear that the ether had a marked effect in A^a, making all of the eggs self-fertilize. This is all the more interesting because none of the eggs without ether self-fertilized. Both eggs and sperm of the B- set appear to have been in poor condition, so that the sperm did not cross-fertilize, or the eggs become cross-fertilized, to any extent.

In searching for other substances that might act on the spermatozoa as does the ether, I tried, amongst other things, a solution of ammonia in sea-water, and this I found made the spermatozoa even more active than the ether. Dilute solutions of alcohol from 1 to 10 per cent. also excite the spermatozoa to greater activity. Certain salt-solutions, ammonium chloride (1, ½, ¼ per cent.), magnesium chloride (2 per cent.), and sodium chloride (1 per cent.) appeared also to act on the sperm, but much less effectively than does ether, alcohol, or ammonia. In the alcohol series of 1, 3, 5, 6, 10 per cent., it was found that 1 per cent. made the sperm very little more active; 3 per cent. more so; 5 per cent. most active; 6 per cent, less; 8 per cent, no effect; 10 per cent., no effect. The last two solutions undoubtedly injured the sperm. In another series, 7 per cent. gave the best results.

A few experiments were carried out in order to see if the sperm made active by the alcohol, would self-fertilize the eggs when it would not do so without the stimulus. Here, as in the preceding series, the same lettering will be used in the tables. A^a self-fertilized in sea-water, A^a self-fertilized in alcohol-solution, A^b crossed in alcohol-solution.

A^a 0	B^b 0
A^a [Alcohol] few	B^b [Alcohol] several
A^b 20	B^a 50

In this experiment while no eggs were self-fertilized in sea-water, a few or several (the percentages were not recorded) were self-fertilized in alcohol, but even more developed in the crossed lots.

In another experiment only one individual was used. The eggs, self-fertilized in sea-water, did not segment, but 10 per cent. did so in a 3 per cent. soluton of alcohol, and 50 per cent. in a 5 per cent. solution of alcohol.

Solutions of ammonia gave similar results. Sperm and eggs were mixed together in very dilute solutions of ammonia. Many eggs divided and of these most appeared, from their method of division into several cells at once, to be polyspermic. Some of the sperm from the last lot was added to eggs in sea-water. Fewer eggs were fertilized, but several that were fertilized were polyspermic. Eggs (not separated from their own sperm) were crossed in ether. All of these were polyspermic. Another set gave almost identical results.

It is clear from these experiments that those solutions that make the spermatozoa more active often induce fertilization of the eggs, when such a fertilization does not take place without the use of the solutions. The activity of the sperm and the fertilization of the egg appear to be directly connected. This point will be more fully discussed later.

EXPERIMENTS WITH MOLGULA.

On each side of the body of Molgula there is an ovary surrounded by a testis. It is very easy to open the central cavity of the ovary, and remove the eggs without cutting the testis.

A few preliminary experiments showed that the sperm of Molgula fertilizes the eggs of the same individual. The following illustrations will show the great powers of self-fertilization of this species:

A^a 85	B^b 90	A^a 100	B^b 2
A^b 90	B^a 100	A^b 100	B^a 90 [irregular
A^a 100	B^b 100	A^a 0	B^b 100
A^b 100	B^a 100	A^b few	B^a 0
	A^a 90	B^b 0[1]	
	A^b 100	B^a 100	

These cases make it clear that the sperm is capable of fertilizing the eggs of the same individual. Whether the sperm of another individual is prepotent I did not attempt to determine. There were only a few cases in which neither self- nor cross-fertilization was effective, and whenever good crossing was accomplished self-fertilization was also realized, showing that when the sperm is good, it will readily fertilize the eggs of the same individual. Since similar results were obtained when three individuals were used it will not be necessary to give the latter cases. The experiments were not extensive enough to show whether good sperm affects the eggs of certain individuals better than it does others, but Molgula is not well suited to test this point.

It occurred to me as possible that in Cynthia and in Molgula the power to self-fertilize the eggs might be due to the eggs coming from the ovary on one side of the body, and the sperm from the other side. Conversely, if this were true, the lack of self-fertilization in Ciona might be connected with the presence of only one ovo-testis. I examined this possibility for Molgula. The eggs from the small ovo-testis were fertilized with sperm from the same side, and other eggs with the sperm from the other side. In both cases all the eggs were fertilized. Conversely, the eggs from the large ovary were fertilized with sperm from the

---

[1] In B the sperm was probably bad. The A^b must therefore have been self-fertilized. The same conditions hold also for the second couple.

same side, and others with sperm from the opposite side. Here also all the eggs segmented. It is perfectly evident, therefore, that the question of self-fertilization in Molgula is not connected with the double condition of the ovo-testis.

EXPERIMENTS WITH OTHER FORMS.

In order to find out how generally ether, alcohol, and ammonia excite to greater activity the movements of cilia, of flagella, and of the spermatozoa of other animals, I made a few experiments on certain protozoa and on the spermatozoa of the frog and of the rat.

Ether, 5 per cent. stops the movements of paramoecium, and kills stentor; 3 per cent. slows up the movements of the former, and causes stentor to throw off its outer layer; the movements of free swimming vorticellae seemed to be increased; 2 and 1 per cent. hasten the movements of paramoecium and of stentor.

Alcohol of 6 and of 8 per cent. slow down the movements of paramoecium and stylonychia, and cause stentor to disintegrate; 10 per cent. kills; 4 per cent. appears to be near the limit, and seems to increase their activity; 2 per cent. clearly increases their activity.

Ammonia 1/200 per cent. kills paramoecium, stentor, and stylonychia; and even 1/2000 also kills; 1/5000 per cent. seems to make these protozoa somewhat more active, but I have not sufficiently tested this solution.

Some of the same solutions were used with euglena, which moves by means of an anteriorly directed flagellum. Ether 5 per cent. makes them somewhat more active; 3 per cent. less so, and 2 per cent. gives no very noticeable effect. Alcohol 10 per cent. kills; 8, 6, and 4 per cent. make them swim more actively; 2 and 1 per cent. give no definite result. Ammonia 1/200 kills; 1/2000 per cent. does not appear to make euglena more active, but other strengths should be tried.

A male spotted frog (*Rana halecina*) was killed in November; its testes opened, and the immobile sperm squeezed out into normal salt-solution. It was found that it took some minutes to get a noticeable effect. Ether 5 and 2 per cent. caused the spermatozoa to show some movement in the course of 15 minutes. Alcohol gave better results. A 10 and a 6 per cent. solution awakened the spermatozoa to activity; a 4 per cent. gave the best results of all. In no case, however, was the activity very great. No movements were detected in ammonia-solutions, but only two strengths were used.

These scattering and incomplete observations show that these substances are in all probability general stimulants for protoplasmic activity of certain kinds.

I have also made a few experiments with the spermatozoa of mice. The spermatozoa were taken directly from the testis of a mouse that had just been killed. The solutions were added to a drop of the sperm squeezed out from the testis into a drop of physiological salt-solution, consequently the dilution is greater than actually given by the percentage. In certain strengths of ether (5 per cent.) and of alcohol (8 per cent.) it appeared that the movement was increased; with ammonia I did not get satisfactory results. The observations are made more uncertain here because, when the testes are opened, spermatozoa in all stages of development are found, and are consequently acted upon differently by the solutions. It would be more satisfactory to use a larger animal and take the spermatozoa from the vasa deferentia, where they are all fully formed. It is certain, however, that alcohol and ether do not produce as great effects on these spermatozoa as they do on the spermatozoa of the ascidians and of some other marine animals that I have examined.

In one of the preparations of the mouse testis the water began to run out at one side and it became apparent at once that the spermatozoa all turned and headed up-stream. It has been recorded by Kraft that spermatozoa swim in the opposite direction to that in which the cilia of the oviducts act. My observation suggests that movement in this direction is not due to the spermatozoa swimming against the direction of the greatest action of the cilia, but against the stream that is produced by the cilia. The movement may be a simple physical phenomenon—the lighter tails of the spermatozoa being swept backwards by the current so that the heads are turned up-stream, and the contraction of the tail then causes the spermatozoön to travel in this direction.

In later experiments the sperm was taken from the vasa deferentia, and put first into distilled water where the spermatozoa remained quiescent. If a drop of salt-solution (water 100, NaCl 0.75) was added to a drop of water containing the spermatozoa, they became active in the course of a minute or less, and their activity continued to increase for several minutes longer, when they remained active for some time. If a drop of a 5 per cent. ether solution is added to a drop of water containing quiescent spermatozoa, no result is seen at first, but after ten minutes I have observed a slight vibration of the spermatozoa. If now after the ether has been added, a drop of the salt-solution is also added, the spermatozoa become active, but it is difficult to determine whether they become more active than when the salt-solution alone is present. Certainly there is no marked difference. If a drop of 8 per cent. alcohol is added to a drop of water containing the spermatozoa no activity is observable, but if then a drop of salt-solution is also added the spermatozoa begin to swim, showing that the alcohol had not injured them, although it had failed to arouse them to activity. Several strengths of KOH (3 per cent. and weaker) were tried, but without effect; yet if salt-solution was added later some slight activity was seen.

In another series of experiments the spermatozoa quiescent in water were first made active by adding the salt solution. If ether was then added no decided effect on the sperm could be seen when their activity was compared with that of check preparations of salt-solution only. It appeared sometimes as though the ether did make the activity more pronounced, and the movement of the spermatozoa appeared somewhat different in the two cases. In the ether the motion was more jerky, and in the salt solution more sinuous and normal.

The following solutions were also tried: The sperm was first put into a drop of water, and then a drop of the solution was added. $NaHCO_3$ 0.625 per cent. caused the sperm to vibrate rather actively; Na_2CO_3, 5.0 per cent. caused a little activity after five minutes; KCl, 0.5 per cent. caused greater activity than did the sodium carbonate, while CaCl caused somewhat less vibration.

These, and some other experiments that need not be described here, show that salt-solutions of various kinds have a marked effect in arousing to activity the inactive spermatozoa of the vasa deferentia. They also make active, spermatozoa that are quiescent in distilled water. On the other hand ether, alcohol, and ammonia, which proved so efficient for the spermatozoa of the sea-urchin and starfish, appear to have little effect on the spermatozoa of the mouse.

The more fundamental physiological question as to the nature of the action of these different substances I shall not attempt to discuss without a further basis of observation and experiment to go upon. Enough has been seen, however, to suggest that the substances act as a "stimulus," which is perhaps not dissimilar in kind from that which causes some eggs to begin to develop, or a nerve impulse to start, or a muscle to contract. Here also we may urge, as I have urged elsewhere[1] in opposition to Loeb's conclusion in regard to the action of certain agents in causing artificial parthenogenesis, that the nature of the stimulus is of such a kind that the result depends much more on the structure or the composition of the living thing than upon the kind of stimulus employed. So unstable is the living organization that the slightest change brought about in it by chemical or by physical means suffices to set into action a perfectly definite and prearranged series of events.

HISTORICAL REVIEW.

The action of ether, ammonia and alcohol on the speramtozoa of Ciona, arousing them to greater activity and thus, under certain conditions, bringing about the fertilization of the egg, raises the question as to whether in the higher animals a similar action may not result from the application of these and of other substances, and also whether the secretions of some of the glands connected with the reproductive system may not have a similar effect on the spermatozoa.

When I tried to find some substances that might bring about self-fertilization in Ciona I was not aware that there had already been made several experiments on the action of solutions on the spermatozoa of other animals. I find that there are quite a number of observations of this sort, although none of the observers have had in view the same question with which I was especially concerned.

---

[1] Science. N. S. XI. 1900. Pp. 178-180.

Kölliker in 1856 carried out an extensive series of experiments on the effect of different solutions on the spermatozoa of the bull, dog, rabbit, horse, and also made a few observations on the spermatozoa from a human cadaver. He found that water alone quickly brings spermatozoa to rest, but does not kill them. They can be aroused to activity by adding, for instance, a 10 per cent. solution of disodium phosphate.[1] Many other substances were found favorable to the activity of the spermatozoa, such as blood-serum, sugar in certain strengths, sodium chloride, caustic potash, etc.

The caustic alkalies (potassium, sodium, and ammonium hydroxide) were found to be especially powerful excitants. Kölliker also tried a number of other solutions, such as three different kinds of sugar, glycerine, gum, etc., which in certain strengths cause increased activity; also urea, gall, morphine, strychnine (nitricum), which have an indifferent effect. He also tried alcohol, creosote, chloroform, ether, alkaloids, and tannin, which have an injurious effect. Kölliker also examined the action of the secretions of the glands of the male reproductive organs—the uterus masculinus, prostate and Cowper's glands. He found that these secretions excite the spermatozoa to greater activity.[2]

The much more recent experiments of Steinach bear even more directly on the present problem. He found that after removal of the glandulae vesiculares ("receptaculum seminalis" of some writers) of the male rat, that, although the sexual instinct remained, the number of young that were born was much decreased. When this gland, as well as the prostate, was removed no young at all were born, although frequent union with the females took place. The results may be due to the semen being insufficiently diluted when it is not mixed with the secretions of the glands, or else to the absence of proper excitation of the spermatozoa when the gland-secretion is removed. That the spermatozoa may be normally acted upon by the secretion of the glands was shown by Steinach in the following way: Sperm from the vas deferens was mixed with a physiological salt-solution. A drop was placed under a cover slip and the edges sealed to prevent evaporation. The preparation was kept at a temperature of 35° to 37° C. A similar preparation was made with the secretion of the prostate. In the former the spermatozoa began to lose their activity in one and a half hours, and after three hours had come completely to rest. In the other preparation, that containing the extract from the prostate gland, the spermatozoa were active after 11 hours, and ceased to move altogether only after 22 hours. This experiment shows that the secretion of the gland prolongs greatly the period of activity of the spermatozoa. Whether it excites them to greater activity is not stated, but Kölliker's results leave no doubt on this score. The decrease in the fertilizing power when the glands were removed may well be connected, as suggested above, with the lessened activity of the spermatozoa.

---

[1] Kölliker gives the formula $2NaOHOPO_5$, which is no doubt disodium phosphate, now written Na_2HPO_4.

[2] Moleschott and Richetti are quoted by Kölliker as recognizing the favorable action of sodium salts on the spermatozoa. Quatrefages found that the spermatozoa of the weasel showed a "surexcitation" in 64 parts sea water to one part sea salt. Newport found that potassium carbonate, and also 1/480 of potassium salt made the activity of the spermatozoa of the frog greater.

Buller has recently studied the question as to whether the spermatozoa of the sea urchin are attracted to the egg,— in other words, whether, as some authors have assumed off-hand to be the case, there is a chemotactic action of the egg on the spermatozoa. He points out that although Strasburger claimed that the egg of Fucus excretes a substance that attracts the spermatozoön from a distance of two diameters of the egg, Bordet and Buller himself have failed to confirm this statement. Massart thinks that in the case of the frog the meeting of the spermatozoön and the egg is purely accidental. Buller finds for the sea-urchins, Arbacia, Echinus, and others, that when the spermatozoa are set free near the egg they show no tendency to swim towards it. The dense collection of spermatozoa that forms around the egg is due to those that happened to run into the jelly sticking there. These spermatozoa then proceed to bore into the jelly; most of them in a radial direction, although a few can be seen to go in obliquely, or tangentially. The same phenomenon occurs in unripe eggs, and in eggs that have been killed in weak osmic acid and the acid washed out. It is improbable, therefore, that chemotaxis has anything to do with the result.

In order to see if any substance is given off by the eggs that attracts the spermatozoön, the eggs were taken from the ovary, carefully washed, and allowed to stand for 2 to 12 hours in a small amount of sea-water. Capillary tubes were then filled with this water and placed in a drop containing spermatozoa. The spermatozoa did not show any tendency to collect around the openings of the tubes. Several other substances were tried in the tubes in the same way,— salts, sugar, ferments, acids, alcohol, etc.—but no chemotaxis was discovered.

The spermatozoa of the sea-urchins swim in spirals. Coming into contact with a surface, the spiral is changed to a circular movement due to contact. Buller considers whether the radial path taken by most of the spermatozoa after they have entered the jelly is due to stereotropism. He reaches the conclusion that while theoretically this assumption will explain the phenomenon, yet conclusive evidence in favour of this view is lacking. He suggests that it may be possible to find a purely physical solution of the problem.

Von Dungern has examined the question of cross-fertilization from the point of view of the different substances contained in the egg, and has reached some conclusions of the greatest interest. He finds that the egg of the starfish, *Asterias glacialis,* contains a substance that acts as a poison on the sperm of the sea-urchin (Echinus or Sphaerechinus). The minimal lethal dose for the sperm mixed with 2 ccm of sea-water varies considerably with the individual; for Echinus between 1/800 to 1/6400 part is fatal in half an hour. Von Dungern tried to obtain an antitoxin from the blood of the rabbit that would neutralize the effect of the poison of the eggs, hoping that it might be possible in this way to bring about the cross-fertilization of the egg of the starfish by the spermatozoön of the sea-urchin. He found, however, that the serum of the normal rabbit already contains a substance that has a powerful antitoxic action on the poison of the starfish, so that it was not necessary to obtain an antitoxin by injecting the poison into the rabbit. The antitoxin of the rabbit's serum was added to water containing the eggs of Asterias, and then sperm from a sea-urchin was supplied. Von Dungern often obtained two- and four-cell stages in this way, but the results were uncertain, and he could not decide whether fertilization had or had not taken place. It seems not improbable, I think, that the outcome may have been due to artificial parthenogenesis which occurs very readily in the eggs of certain starfish; in fact, it is very difficult to prevent its occurrence, unless the eggs are very carefully handled.

The same poison that is present in the eggs of the starfish is also secreted by the skin. It is also rendered harmless by the rabbit's serum. In the sea-urchin there is a poisonous substance in the gemmiform pedicellariae, which is very injurious to the sperm of the starfish. If 100 of the pedicellariae of Sphaerechinus are rubbed up in one ccm of sea-water, the solution will destroy in a quarter of an hour the sperm contained in ten to twenty litres of sea-water. The minimal lethal dose for 2 ccm is 1/5120 to 1/16240 ccm. The spermatozoa of Sphaerechinus itself are killed by this fluid, but a much stronger dose is necessary. On the other hand an extract of the egg of Echinus, Sphaerechinus, Strongylocentrotus, or Arbacia does not kill the spermatozoa of the starfish even in the strongest solutions. What then prevents the spermatozoa of the starfish from entering the eggs of these sea-urchins? There is another factor, Von Dungern thinks, that interferes with this combination. The egg membrane of these urchins has an agglutinizing effect on the spermatozoa of the starfish. This agglutinizing effect appears to be the same phenomenon as that seen "whenever cells of any kind are introduced into the body of another animal." So far as this process is involved in the union of germ-cells, Von Dungern thinks that under certain conditions it might assist the fusion, while under others it might interfere with it. Thus two naked and equivalent cells might be helped to unite, while an egg surrounded by an agglutinizing jelly would fail to be fertilized. The substance in the sea-urchin's egg that agglutinizes the starfish sperm can be rendered ineffective by the rabbit's serum. Not all starfish spermatozoa are agglutinized by the jelly or by the egg-substance of all the different sea-urchins. In Sphaerechinus it fails to occur. Therefore in this case the failure to cross-fertilize must be due to some other factor, and, in fact, Von Dungern claims to have found still another substance in the sea-urchin's egg that excites to greater activity the immature and quiescent spermatozoa of the starfish. These immature sperm, made active by this substance, are then capable of fertilizing the eggs of the starfish. He found that weak doses of chloral hydrate and of cocaine also make these quiescent spermatozoa active, and that rabbit's serum has a marked effect. Von Dungern believes further that these exciting substances may actually prevent, in certain cases, the cross-fertilization, because they may change the kind of reaction shown by the sperm. He observed that the spermatozoa of those species that do not normally show rotational movements when they come in contact with surfaces, usually do so when the exciting substances just mentioned are present. It does not appear to me, however, that this is an altogether satisfactory explanation of the failure of cross-fertilization in these cases.

Von Dungern also examined the question as to whether the egg secretes a substance that favours fertilization by its own sperm. He believes that he has also discovered such a substance. The eggs of Echinus (or of Sphaerechinus) are rubbed up and mixed with pieces of jelly that have been carefully washed. When sperm is added to the water in which such pieces lie they stand vertically to the surfaces of the pieces. If on the other hand the pieces of jelly are not mixed with the substance from the egg, the spermatozoa simply rotate on the surface of the jelly, and do not stand vertically. Starfish spermatozoa with Arbacia jelly behave as with simple jelly alone, *i. e.*, they do not stand vertically. The vertical position of the spermatozoa is due, Von Dungern thinks, to the presence of some substance in the extract that lowers the excitability of the spermatozoön to contact, and hence it takes a vertical position. He also points out that this same substance causes the spermatozoa to lose their power of movement in a short time. Thus, while Von Dungern finds no evidence of a substance in the egg that attracts the sperm, he believes that there may be present in some eggs a substance that favours the fertilization of the egg, by causing the spermatozoön to assume that position in the jelly that is most likely to bring them to the surface of the egg.

Loew has attempted to show by an experiment, which is not, I think, well suited to prove his point, that the spermatozoa of the rat are attracted to, *i. e.*, that they are positively chemotactic to, the slime layer of the uterus and also to the alkaline mucosa of the digestive tract, but not to the acid slime of the vagina. His method of experimenting was as follows: A piece of the mucosa of the uterus was put on one side of a slide and a piece of the vagina on the other. A drop containing the sperm was placed in the middle of a cover-slip, and this put over the pieces on the slide. It was found that the sperm collected more on the side near the piece of the uterus, and from this Loew infers that they have been attracted to this side. In the light of the other experiments described above it will be clear, I think, that the greater accumulation of the sperm on one side by no means establishes the conclusion that they have been attracted to this side. Loew tried to show that filter paper saturated with alkaline substances acts chemotactically on the spermatozoa, in the sense that they move towards such substances, but, as in the preceding case, it does not necessarily follow because spermatozoa collect around or in certain substances, therefore they must have moved towards these substances. The recent work of Jennings on the protozoans shows that their accumulations in certain areas is not due to the action of substances that cause the individuals to swim towards those substances, but on the contrary to their action being such that those individuals that enter areas containing these substances are unable to leave them. The *result* is the same as when the spermatozoa touch the jelly of the egg and stick to it, although the *means* by which the accumulations are formed in the two cases are entirely different. It would be interesting to see if spermatozoa may not behave towards certain solutions as do the protozoans.

THEORETICAL.

It has been often assumed by embryologists that there exists some sort of attraction between the eggs and the spermatozoa of the same species. This idea would readily suggest itself to anyone who saw spermatozoa collecting in crowds around the eggs, but it by no means follows that this phenomenon is really due to an attracting substance emanating from the egg. The result may be due to the membrane of the egg, to which those spermatozoa stick that come accidentally into contact with it. In fact I have observed similar collections of spermatozoa in the ascidian around pieces of the body tissue, where the result had every appearance of being due to some sticky substance, exuding from the piece, rather than to an attraction exerted by the piece on the spermatozoa.

Pfeffer's oft-quoted experiment with the antherozoöids of ferns, liverworts, etc., appears to support the idea that the antherozoöids are attracted to the malic acid that is present in the neck of the archegonia, but in the light of the recent experiments of Jennings and others, as to the way in which unicellular forms accumulate in a drop of acid, we can readily see that the results may have a very different interpretation from that usually given to them. Confining our discussion to the results obtained with the ascidians, I offer the following tentative analysis of the problem:

The failure of the spermatozoön of Ciona to enter the egg of the same individual may be conceived as due to some physical obstacle. It is conceivable that pores may exist in the egg-membrane, or even in the surface of the egg itself. This is the argument that Pflüger[1] used in the case of cross-fertilization of the frog's egg. If in the ascidian there existed a correlation of such a sort, that the size of a spermatozoön of a given individual is always greater than the pores of the eggs of the same individual, then self-fertilization could not take place. That this is not the real explanation is shown by the fact that good spermatozoa are apparently capable of fertilizing the eggs of all other individuals. This would certainly not be the case if the exclusion of the spermatozoön from the egg of the same individual was due to the size of the pores, because there would be eggs of some other individuals having pores as small or smaller. Another possibility that suggests itself is that the surface tension of the egg is of such a sort that it excludes the spermatozoa of the same individual, but this idea does not appear to give a satisfactory solution, for, aside from the fact that it is difficult to imagine how such a relation could exist, there would also occur cases in which the surface tension of the eggs of other individuals would exclude certain sperm, and this does not appear to be the case. It is true that the addition of the ether to the water may cause a difference in the surface tension of the egg, and it might be made to appear that this was the way in which the self-fertilization is effected in the ether-solutions, but I can not believe that this is the explanation of the results, because other experiments show that a considerable amount of ether is necessary to cause self-fertilization.

---

[1] Archiv. f. die gesammte Physiologie, XXIX., 1882.

It seemed to me that violent shaking might so affect the surface of the egg that self-fertilization might take place. A number of eggs from the oviduct were violently shaken for a few minutes in a small vial, and then sperm from the same individual was added. No segmentation took place, and the presumption is therefore that the eggs were not fertilized.

Turning to the chemical side we find a number of possibilities that demand consideration. The inactivity of the immature spermatozoa, and the lack of power of such sperm to fertilize the egg, their becoming active in certain solutions, and their power then to fertilize eggs that they did not fertilize before, as best shown in Cynthia, suggests that normally the eggs may secrete certain substances that make more active the spermatozoa, which then become capable of fertilizing the eggs. This view appears all the more attractive in the present case on account of the observed lethargy of the spermatozoa of these ascidians, and the apparent connection in such cases between this condition and the impotence of such sperm in fertilization. Yet after careful consideration I am not prepared to advocate this view as the only solution, although I realize that it might be made to give the appearance of a ready explanation of my results. Not that this induced activity may not be one of the factors to be taken into consideration, but it is not, I think, the whole explanation. My reasons for regarding this view as insufficient are the following: It was found that sperm that appeared to be very little active was sometimes capable of cross-fertilizing the eggs of another individual. Possibly this may be due to somewhat greater activity induced by something secreted by the eggs of the other individual, yet on the whole I can not claim that direct observation gave any convincing evidence in favour of this assumption. More significant are the results of the experiment of mixing eggs from two individuals, and subsequently fertilizing them with the sperm from one of the individuals. Half only of the eggs segmented, presumably those cross-fertilized. If some substance that makes the sperm active were really thrown out by the eggs, then we should expect that all the eggs would have been fertilized, unless indeed the secretion loses its power a short distance from the surface of the egg that secretes it; but this does not seem to be a probable interpretation.

A different point of view is that the egg secretes some substance that attracts the spermatozoa. On this view we must suppose that the substance secreted by the egg of Ciona has no attraction for the spermatozoa of the same individual.

The little evidence that I have to offer, based on experiments with ascidians, is not favorable to this idea, that the cross-fertilization is due to some attractive substance secreted by the egg. In the species that I have examined there is no such marked accumulation of spermatozoa around the eggs as is seen in many other animals, and nothing in the behaviour of the cross- and self-fertilized egg to suggest that the difference in the results is due to an attraction in the one case, and to the absence of an attraction in the other. In other forms where there is a better opportunity for examining this question the most recent observations go to show, as has been pointed out in detail above, that there is no sufficient evidence for the view that the egg attracts the spermatozoön.

Conversely, it may be supposed that the egg secretes some substance that *repels* the spermatozoa of the same individual. I observed nothing that would support such a conclusion, and this interpretation of the process would be foreign to what we find in general in connection with fertilization even in cases where the sperm of one species does not fertilize the eggs of another.

We come now to a more subtile argument, and one that we are scarcely in position to discuss profitably in our present state of ignorance concerning the union of egg and spermatozoön. It may be assumed that there is some sort of "chemical affinity" between the egg and the spermatozoön that causes the two to unite when they come together. On this assumption we should have to suppose in Ciona that this affinity does not exist, or at least is less strong, between the egg and the spermatozoa of the same individual than between those of different individuals. Such a statement carries us no further, however, than the facts, and in the case of Cynthia we should have to assume that the affinity is so nicely balanced that sometimes the spermatozoön can unite, and sometimes it can not. In the case of Molgula the affinity must be assumed to suffice to bring about self-fertilization. Until we can give some more tangible form to this idea it does not appear to have any greater value, than the mere statement of the facts, and indeed may have less value, since it may give a wrong impression as to the real factors at work.

Finally there might be advanced what may be called the electrochemical hypothesis. The union of the egg and the spermatozoön may be supposed to be an electrical phenomenon, connected with a difference in the chemical composition of the two elements. The sperm head is almost pure nuclear chromatin, while the surface of the egg is protoplasmic. Possibly the spermatozoön and the egg have different electrical charges and unite with each other if brought near enough for the charges to become effective. But on this supposition it is not clear why the eggs and the sperm of the same individual would not unite. Here also we get no light on the absence of self-fertilization in Ciona.

I have kept constantly in mind while at work on this problem the possibility that the spermatozoön may really enter the egg, but fail to develop there, or fail to start the development of the egg, because, coming from the same individual, it was not sufficiently different in composition to supply the necessary stimulus. The ether might be supposed to make the sperm sufficiently different from the egg to start the cleavage, or the ether might itself supply the stimulus which is capable of starting the development of the egg *after* the spermatozoön has entered.

The test of this view should be found in direct observation of the eggs themselves. I prepared therefore a series of eggs of Ciona, some unfertilized for check series, others self-fertilized, but not put into ether, and others like the last, but put into ether.

The difficulties of determining whether the spermatozoa can enter the eggs of the same individual, but fail to start the development, are greater than may appear at first sight. The sperm head is so minute that if after it entered no changes were affected in the protoplasm about it, its presence might be readily overlooked, and since the spermatozoön of Ciona enters the egg in a granular zone that colors more deeply in certain stains than does the rest of the egg, the difficulty is thereby increased. Of course I have been on my guard against cases where the surrounding sperm have floated over the section, as sometimes happens, or have been carried over it by a defect in the knife, and I have also been careful to exclude all cases where specks of foreign matter may have been on the slide, or in the fixative. There are also two further precautions to be taken. When the egg withdraws from the membrane and the test-cells are extruded, as it were, from the outer zone of the egg, the protoplasm is sometimes drawn out in mamiliform processes that stain deeply and resemble the entrance cone formed by the spermatozoön penetrating certain eggs. Even when the protoplasm does not protrude, deeply staining spots are generally present and are especially obvious after iron haematoxylin. Careful staining with Delafield's haematoxylin shows clearly that these spots have nothing to do with the entrance of spermatozoa. Furthermore these spots are found in unfertilized eggs. After iron haematoxylin minute deeply staining bodies, flattened against the outer surface of the egg, can generally be found, and these strongly suggest spermatozoa. That they are not such is shown by their presence in unfertilized eggs, and by their absence after the Delafield stain. I mention these points because they might easily lead one after only a casual examination to conclude that spermatozoa enter the eggs. My best results have been obtained by drawing out the iron haematoxylin until the protoplasm has lost all of its color, or better still by using the Delafield stain, and also thoroughly extracting the color from the protoplasm.

Although I have examined a large number of preparations I have not seen a single definite case without ether in which a spermatozoön has entered the egg of the same individual. Difficult as it admittedly is to be absolutely certain on this point, yet if the spermatozoa had entered and had begun to enlarge I feel certain that I should have detected their presence. That undeveloped sperm-heads may be present I must admit as a possibility, but I have not detected them, and believe that I should have been able to do so were they present. It is also a point of some importance that I have not found any spermatozoa within the egg membrane, although quantities of them may lie outside.

There is a further point in this connection, the importance of which I did not appreciate until I had closed the experimental part of my work. In the eggs of many animals a change takes place in the egg, after the penetration of one spermatozoön, of such a sort that the entrance of more spermatozoa is prevented. I have found in Ciona that, after the sperm has stood with the eggs of the same individual and has failed to fertilize them, these eggs could still be readily fertilized by spermatozoa from another individual. If a spermatozoön of the same individual really enters the egg it does not in consequence bring about such a change in the egg that other spermatozoa can not enter, and therefore many spermatozoa of the same individual from which the eggs were taken should be expected to gain entrance, but I am quite certain that this, at least, does not occur. From this consideration also it may be inferred that the spermatozoa do not normally penetrate the eggs of the same individual.

In the light of these observations it seems probable that whenever a spermatozoön enters the egg, the egg begins to develop regardless of whether the spermatozoön comes from the same or from another individual. The ether must therefore induce a change of some sort that directly effects the entrance of the spermatozoön into the egg, and at present I see no other interpretation that is left than that this entrance is due to the greater activity of the spermatozoön that causes it to overcome some resistance, either on the surface of the egg itself, or in the membrane surrounding it. The nature of this resistance I did not detect, and this must be the next step in the analysis. One method by which this view may be tested is obvious, and has already been referred to. The spermatozoa made active by sea-water must be placed in an extract of the eggs (or body-tissues) of the *same* individual, and then, after a time, the eggs of another individual added. On the hypothesis these eggs *should be less likely* to become fertilized than eggs placed directly in contact with the fresh sperm.

It has been found that certain substances secreted by the glands of the reproductive organs of the male mammal arouse the spermatozoa to greater activity. It has also been found that many other substances have a similar effect on spermatozoa. It would be equally interesting to discover if the secretions of other parts of the genital ducts of the male or of the receptacula of the female, when such are present, may not bring the spermatozoa to rest, or keep them quiescent until some other exciting agent arouses them. It seems almost certain that this must be the case in those animals in which the spermatozoa of the male are stored up in receptacula of the female, as for instance in the honey bee, or in such a hermaphroditic animal as the earthworm. The length of life of the spermatozoa in some of these forms would seem to make some assumption of this sort necessary. Experiments can easily be made that would decide this question. Kölliker has shown, in fact, that water quiets the spermatozoa of mammals without killing them.

In the ascidians it is probable that the spermatozoa in the vas deferens are quiescent. It is significant that in these hermaphroditic forms the oviduct in which the eggs are stored takes a course parallel to the male duct. Possibly the proximity of the two ducts may be connected with the lack of power of self-fertilization of the eggs, because the egg may be saturated with the same substances that keep the sperm quiescent. It may be, however, that this relation is more fundamental, and the particular substance is one peculiar to the whole body. That the reaction must be something quite specific is shown by the fact that the spermatozoa are able to enter eggs of any other individual.

It appears probable that of all the different substances that excite the spermatozoa to activity the secretions of the glands connected with the male reproductive organs may be the most efficient. From a statement of Kölliker's it seems not improbable that the substance secreted in the glands of one species may be also efficient for the spermatozoa of other species. Whether by the use of the substances from the glands of another mammal it might not be possible to excite human spermatozoa to greater activity and thus assist materially in bringing about fertilization in cases where the impotence is on the side of the male remains to be examined. There is here a question that may have an important practical aspect.

The lack of power to self-fertilize in plants may also be due to the inability of the pollen tube to penetrate sufficiently far into the stigma and style. It appears that penetration does actually begin in some cases that have been observed, but possibly the growth may be arrested further down in the style. The prepotency of other pollen would then find its explanation in the more rapid growth of this foreign pollen. Here again is an opportunity for future work.[1]

In attempting to formulate a theory to account for the determination of sex, Castle assumes that there are two kinds of spermatozoa, male and female, and that there are also two kinds of eggs, male and female. He also assumes that a female egg can be fertilized only by a male spermatozoön and that a male egg only by a female spermatozoön. I have already pointed out elsewhere[2] that my results do not support this assumption. Castle appealed to the case of Ciona as one in favour of his contention, for the eggs here can not be fertilized by the sperm of the same individual. It is not explicitly stated to the contrary, and the reader might be led to infer from the context that in Ciona all the eggs and all the spermatozoa of one individual must be supposed to be male, and in another individual the reverse; but certainly this is not the case, and could not have been Castle's meaning, for if it were so then half of the individuals would be infertile with the sperm of the other half, and this is not so. I have pointed out that my results with ether, etc., do not support Castle's assumption, although it might, of course, be claimed that the ether causes the spermatozoa to lose, as it were, their homosexual repugnance. However this may be, I have found that no such lack of power to self-fertilize is found in some other ascidians, as in Molgula for example. If my supposition is correct, that self-fertilization in Ciona is due to the presence in the eggs of some substance that brings the spermatozoa to rest, the whole question assumes a very different aspect and does not appear to have any connection with the question of the determination of sex.

---

[1] The experiments of Myoshi should be especially considered.

[2] Popular Science Monthly, Dec., 1903.

LIST OF REFERENCES.

BORDET. — Contribution a l'Etude de l'Irritabilité des Spermatozoïdes chez les Fuccacées. Bull. de l'Acad. Belgique 3*me*. ser. XXVII, 1894.

BULLER, A. H.—The Fertilization Process in Echinoidea. Report 70, Meeting Brit. Assoc., 1901.

Is Chemotaxis a Factor in the Fertilization of the Eggs of Animals Quart. Journ. Micro. Sc., XLVI. 1903.

BULLER, A. H. R.—Contributions to our Knowledge of the Physiology of the Spermatozoa of Ferns. Ann. of Botany, XIV. 1900.

CASTLE, W. E.—The Heredity of Sex. Bull. Mus. Comp. Zoöl., XL. 1903.

DEWITZ, J.—Über Gesetzmassigkeit in der Ortsveränderung der Spermatozoën und in der Vereinigung derselben mit dem Ei. Archiv. f. die gesammte Physiologie. XXXVIII. 1886.

v. DUNGERN, E.—Die Ursachen der specifität bei der Befruchtung. Centralbl. f. Phys. XIII. 1901.

Neue Versuche zur Physiologie der Befruchtung. Zeitschr. f. allgem. Physiologie. I. 1902.

IVANCFF.—Journal de Physiologie et de Pathologie general. II. 1900.

KÖLLIKER, A.—Physiologische Studien ueber die Samenflussigkeit. Zeitschr. f. wiss. Zool. VII. 1856.

KRAFT, H.—Zur Physiologie des Flimmerepithels bei Wirbelthieren. Archiv. f. d. gesammte Physiologie. XLVII. 1890.

LIDFORSS.—Über den Chemotropismus der Pollenschlauche. Ber. d. Deutsch. Bot. Gesell. XVII. 1895.

Loew, O.—Die Chemotaxis der Spermatozoën im weiblichen Genitaltract. Sitzungsber. d. Wiener Akad.; Math.-naturw. Cl. CXI. 1903.

Massart, J.—Sur l'Irritabilité des Spermatozoïdes de la Grenouille. Bull. de l'Acad. roy. de Belgique. 3*me* Sér. XV. 1888.
Sur la Penetration des Spermatozoides dans l'Oeuf de la Grenouille. Bull. de l'Acad. roy. de Belgique. Zme. Sér. XVIII. 1889.

Miyoshi, C.—Über Reizbewegungen der Pollenschläuche. Flora. LXXVIII. 1894.

Molisch, H. — Über die Ursachen der Wachtumsrichtunger bei Pollenschläuchen. Sitzungsber. d. k.·Acad. d. Wiss. in Wien. 1889, 1893.

Morgan, T. H.—Recent Theories in Regard to the Determination of Sex. Popular Science Monthly. Dec., 1903.

Pfeffer, W.—Locomotorische Richtungsbewegungen durch chemische Reize. Untersuchungen aus d. Bot. Inst. zu Tübingen. I. 1884.

Steinach, E.—Untersuchungen zur vergleichenden Physiologie der männlichen Geschlechtsorgane insbesondere der accessorischen Geschlechtsdrüsen. Archiv f. d. gesammte Physiologie. LVI. 1894.

Strasburger, A.—Das botan Prakticum, 2 Auf 1887. Page 402.

NOTES ON REGENERATION IN TUBULARIA CROCEA.

HELEN DEAN KING.

The following experiments on *Tubularia* (*Parypha*) *crocea* were made during the summer of 1903, at Woods Holl, Mass., while I was occupying a research room of the Carnegie Institution in the Marine Biological Laboratory. The work was done under the direction of Prof. T. H. Morgan to whom I am indebted for many helpful suggestions.

I. THE EFFECT OF THE EARLIER CLOSING OF ONE END OF A LONG PIECE OF THE STEM OF TUBULARIA.

In experimenting on the European hydroid *Tubularia mesembryanthemum*, Morgan (10) allowed the ends of long pieces of the stem to close and then, after an interval of from one to eight hours, he cut the pieces transversely through the middle region so that two new cut surfaces were exposed (Fig. 1, *B*, *C*). As a result, the aboral development of the proximal piece *CD*, was hastened. In many cases a polyp appeared on the aboral surface, *D*, as soon as did a polyp on the oral end, *C*, and in a few pieces a hydranth developed at *D* as early as did the hydranth on the distal end of the anterior piece, *AB*. This result is explained by Morgan as follows: "When a piece is cut in two in the middle one, two, three or more hours after its ends have closed, the influence of the oral end is temporarily removed, and the aboral end, which now has a start on the new oral end, may gain the ascendency and be the first to produce a polyp. Often, however, the polarity of the piece is sufficiently strong to give the precedence to the influences acting on the oral end. When the two influences are equally balanced, two hydranths may simultaneously develop."

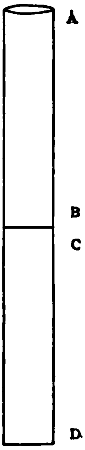


FIG. 1.

In repeating these experiments on the American hydroid, *Tubularia crocea*, the hydranths already present at the anterior ends of the stems were removed by a transverse cut about 2 mm. behind the proximal circle of tentacles, and then pieces of stem varying in length from 30–40 mm. were cut off and the ends allowed to close. After an interval of from two to eight hours the stems were cut through the middle as in Fig. 1, *B*, *C*, thus producing a freshly cut surface (Fig. 1, *B*) at the aboral end of a distal piece, and also one at the oral end (Fig. 1, *C*) of a proximal piece. The results of this series of experiments are given in Table I. to IV. The first column gives the total number of individual stems operated upon; the second column shows the time that elapsed between the removal of the hydranth and the cutting of the stem through the middle; and in the following columns the results two, three, and four days after the operation are indicated. *Hy.* signifies the regeneration of a complete hydranth; *t. a.* indicates the formation of tentacle anlagen only; while *O* is used to indicate that no regeneration had taken place when the observations were made. The letters *A*, *B*, *C* and *D* refer to surfaces thus marked in Fig. 1; and the numbers in parentheses show the number of cases in which similar results were obtained.

TABLE I.

Number of Individuals.	Interval Between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
6	2 hours.	*A* ...*hy.* (6)	*A* ......same.	*A* ......same.
		B... *O* (6)	*B*	*B*
		C.... *t. a.* (3) *O* (3)	*C*.... *hy.* (4) *t. a.* (2)	*C*......*hy.* (6)
		D... *O* (6)	*D*... *t. a.* (2) *O* (4)	*D*... *hy.* (2) *t. a.* (1) *O* (3)

Owing, probably, to differences in the temperature of the water in which they live, regeneration in *Tubularia crocea* is much slower than in *Tubularia mesembryanthemum*. In the former species a new hydranth rarely develops until two days after the removal of the old hydranth, while in the latter species a new hydranth frequently regenerates in the course of twenty-four hours. In all of the experiments in this series, as shown in the above table, a polyp formed on the oral end, *A*, of the anterior piece, *AB*, before one developed on the oral end, *C*, of the proximal piece, *CD*. This result might, of course, be expected as the oral end, *A*, closed two hours before the end, *C*. In no case did a polyp develop at the aboral end, *D*, of the proximal piece as soon as one formed at the oral end, *C*. A start of only two hours, however, is sufficient, in most cases, to cause the formation of a hydranth at *D* before one develops at the aboral end, *B*, of the distal piece, *AB*.

The results obtained when the intervals between the cuttings were four, six and eight hours are given in Tables II. to IV.

TABLE II.

Number of Individuals.	Interval between Cuttings	Result in Two Days	Result in Three Days.	Result in Four Days.
10	4 hours.	*A* ... *hy.* (6) *O* (4) *B* ... *O* (10) *C*.... *t.a.* (1) *O* (9) *D* ... *O* (10)	*A* ... *hy.* (6) *t.a.* (4) *B* ... *O* (10) *C*.... *hy.* (2) *t.a.* (5) *O* (3) *D* ... *t.a.* (1) *O* (9)	*A* ... *hy.* (10) *B* ... *t.a.* (2) *O* (8) *C*.... *hy.* (7) *t.a.* (3) *D* ... *hy.* (1) *O* (9)

TABLE III.

Number of Individuals.	Interval between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
8	6 hours.	*A* ... *hy.* (3) *t.a.* (3) *O* (2) *B* ... *O* (8) *C*.... *hy.* (1) *O* (7) *D* ... *O* (8)	*A* ... *hy.* (6) *t.a.* (2) *B* ... *O* (8) *C*.... *hy.* (5) *t.a.* (3) *D* ... *t.a.* (4) *O* (4)	*A* ... *hy.* (8) *B* ... *O* (8) *C*.... *hy.* (8) *D* ... *hy.* (4) *O* (4)

TABLE IV.

Number of Individuals.	Interval Between Cuttings.	Result in Two Days.	Result in Three Days.	Result in Four Days.
12	8 hours.	*A* ... *hy.* (6) *t. a.* (4) *O* (3) *B* ... *O* (12) *C*.... *O* (12) *D* ... *O* (12)	*A* ...*hy.* (12) *B* ...*O* (12) *C*.... *hy.* (10) *t. a.* (2) *D* ... *hy.* (2) *t. a.* (3) *O* (7)	*A* ...*hy.* (12) *B* ...*O* (12) *C*....*hy* (12) *D* ... *hy.* (5) *O* (7)

There is a great similarity in the results of these experiments. In all cases a hydranth formed on the oral end, *A*, of the anterior piece before one developed at any other cut surface, and the rate of development of a polyp from the aboral surface, *D*, was more rapid than that from the aboral surface, *B*, even in those cases in which *D* had only two hours start over *B*. In no case, however, did a polyp form at *D* before one developed at *C*, even in the experiments in which *D* closed eight hours before *C*. As was the case in the experiments made by Morgan, an interval of eight hours between the two cuttings has, apparently, no more effect on the regeneration than has an interval of only two hours. The formation of a polyp at the oral end, *C*, of the proximal piece, *CD*, does not prevent the early development of a polyp at the aboral end of the same piece, and in some cases there is only a few hours interval between the formation of the two hydranths. The earlier development of a hydranth at *A*, however, seems to check the formation of a hydranth at the aboral end, *B*, of the distal piece for some time, as in no case did a hydranth develop at *B* until five days after the experiment began. This difference in the rate of development at *D* and at *B* cannot be due to a difference in the lengths of the pieces, because, in making the experiments, the stems in all cases were cut as nearly as possible through the middle and any difference in the lengths of the anterior and of the proximal pieces would be too slight to have any appreciable influence on the result. The earlier closing of the aboral end, *D*, of the proximal piece, *CD*, evidently counterbalances to some extent the influence of the oral end, as suggested by Morgan. As a result, the development of a polyp at *D* is hastened somewhat, although in no case is a hydranth formed here before or as soon as one develops at the oral end of the piece.

The effect of the earlier closing of the aboral end of long pieces of the stem, in both *Tubularia mesembryanthemum* and in *Tubularia crocea*, is to hasten the development of the aboral surface. The influences that bring about this result are apparently not as strong in the latter species as in *Tubularia mesembryanthemum* where the aboral development may be hastened so much that polyps develop simultaneously at both ends of the piece.

This seeming difference between the two species may possibly be due to the fact that *Tubularia crocea*, which lives in cold water, regenerates very slowly and, therefore, comparatively slight differences in the rate of regeneration at the oral and aboral ends of the stem can be readily noted. *Tubularia mesembryanthemum*, on the other hand, lives in much warmer water and its regeneration takes place so quickly that it is difficult to detect slight differences in the rate of development of the polyps at the cut oral and aboral surfaces.

In a variation of the above experiment, a piece of silk thread was tied tightly around the stem about 2 mm. below the hydranth, and another piece was tied about 30–40 mm. below the first. Both ends of a long piece of stem were, therefore, closed at practically the same time in such a way that no regeneration was possible from either end of the piece. After the ends had been tied, the stem was cut transversely through the middle as in Fig. 1, *B*, *C*, in order to ascertain whether subsequent regeneration from the cut surfaces, *B*, and *C*, would be hastened in comparison with the rate of regeneration from similar surfaces of pieces of stem of the same length, cut at the same time, but not closed artificially at one end. The control pieces of stem were kept in the same dishes with those used in the experiment, and both sets, therefore, were under the same external conditions.

Eight long pieces of stem were used in this experiment. Two days after the operation, tentacle anlagen had appeared at the cut ends of all of the sixteen pieces, but they were not as well developed on the aboral end, *B*, of the anterior piece as they were on the oral surface, *C*, of the posterior piece. At this time there was no indication of any regeneration at the aboral surface of the anterior piece in the control set of stems, although in some cases complete hydranths, in other tentacle anlagen, were present on the oral end of the proximal pieces. On the third day after the operation, polyps were found on the oral end, *C*, of all of the proximal pieces, both in the control and in the tied stems. The development from the aboral surface, *B*, of the anterior pieces, however, did not keep pace with that at the oral end, *C*, of the proximal pieces, as at this time only two of the pieces of stem tied at one end had produced hydranths at the aboral surface, *B*, the rest had, as yet, developed only tentacle anlagen; in the control stems, no development from the aboral surface, *B*, had taken place in any case.

It is seen from the above experiments, that the development of a hydranth at the oral end of a piece of the stem of *Tubularia crocea* is not hastened by artificially closing the aboral end. Tying the oral end of a distal piece of the stem, however, hastens the development of the aboral end of the piece as compared with the development that takes place from the aboral surface of a piece of stem of similar length that is not closed at the oral end, as Driesch has shown. This result also agrees with that obtained by Loeb (7) in experiments in which he stuck the oral end of pieces of the stem of *Tubularia mesembryanthemum* in fine sand, leaving the other end freely surrounded by water. He found that "Durch Hemmung der Polypenbildung am oralen Ende kann man also die Polypenbildung am aboralen Ende beschleunigen."

In a third set of ten experiments, hydranth bearing stems about 30 mm. in length were removed from the colony and kept until a polyp formed on the cut aboral end. The time required for the development of these aboral polyps varied from three to five days in different cases. After all of the pieces had developed hydranths at the aboral end, each stem was cut transversely through the middle as in Fig. 2, *B*, *C*. The object of these experiments was to ascertain whether the presence of a hydranth at the aboral end, *D*, of the proximal piece, *CD*, would alter the polarity of the piece and thus prevent or retard the development of a hydranth at the oral end, *C*. The stems were cut through the middle region on June 19. Not until June 22 were there any indications of a development of a hydranth at the oral surface, *C*, and then only faint traces of tentacle anlagen were found in three stems. At this time, no development had taken place from the aboral surface, *B*, of any of the anterior pieces, *AB*. For control experiments, pieces of stem about 30 mm. in length were cut through the middle as in Fig. 1, *BC*, at the same time that the transverse cuts were made across the stems bearing a hydranth at each end, and on June 22, well developed hydranths were present at the oral end of all of the proximal pieces. On

June 23, three of the ten proximal pieces of stem bearing aboral hydranths had also developed hydranths at the oral end; while the oral end of two other pieces of stem showed well developed tentacle anlagen which developed into hydranths on the following day. No further changes took place in any of the pieces although they were kept for some ten days longer.

It is seen from the above experiments, that the presence of a hydranth at the aboral end of a piece of the stem of *Tubularia* delays, but does not prevent, the development of a hydranth at the oral end of the piece. This result cannot be due simply to the fact that the proximal end of the piece was closed by the presence of the aboral polyp, because, in the previous set of experiments, it was shown that closing the aboral end of a piece of stem by tying does not delay the development of a polyp at the oral end. It seems probable that the polarity of the piece was changed, for a time at least, by the presence of a hydranth at its aboral end and, therefore, the influences for hydranth formation at the freshly cut oral surface were not strong enough to bring about the development of a polyp for some days.

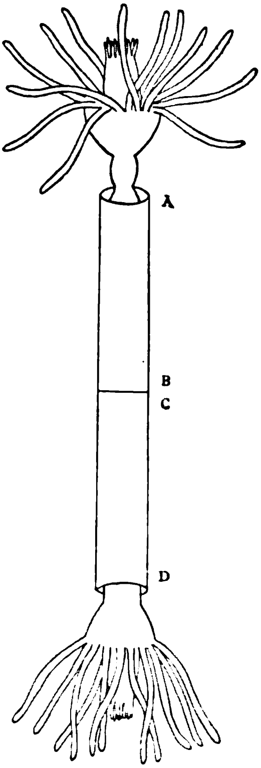


Fig. 2.

II. Experiments on Branching Stems.

The following series of experiments were made in order to ascertain whether the development of a hydranth on the oral end of a stem will influence the rate of development of a hydranth on the distal end of a long or of a short piece of a branch, and also to determine what conditions are necessary in order that the formation of a hydranth at the one place will prevent the formation of a hydranth at the other.

Series 1.—On June 24, twenty experiments were made in which a branch was cut off about 1 mm. from its origin in the stem, and then the anterior end of each stem was removed by a transverse cut leaving a piece from 10–20 mm. above the place of union with the branch (Fig. 3). In one case, a hydranth developed on the cut end of the branch two days after the operation, and at the same time a polyp also formed on the oral end of the main stem which in this instance was 20 mm. in length above the origin of the branch. In all other individuals at this time tentacle anlagen had formed at the oral end of the stem, but there was no indication of the development of a hydranth at the cut end of any of the branches. On June 27 hydranths were found at the oral end of all of the stems and also on the distal end of four branches; all the remaining branches had well developed tentacle anlagen excepting one which showed no signs of regeneration during the course of the week that the hydroids were kept. In this set of experiments, therefore, with the exception of the one case noted, regeneration of a hydranth took place at the distal end of the long stem before a polyp formed at the oral end of the short branch.

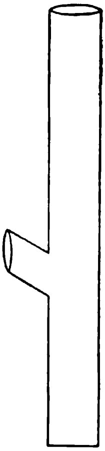


FIG. 3.

The results of this set of experiments might possibly be considered to be due to the fact that the longer piece exerted some kind of an influence over the shorter piece that would tend to alter the polarity of the shorter piece and thus retard development from its cut oral surface. That a larger piece of a hydrozoa can influence the polarity of a smaller piece is shown unquestionable in grafting experiments that I made on *Hydra viridis* (King, 6) in which the larger component of the graft either absorbed the smaller component or formed a permanent union with it. In the latter case, the polarity of the smaller piece was completely reversed, if necessary, in order that a structure might regenerate on its cut surface that would produce a normal polyp. Another factor that might, possibly, cause a delay in the development of a hydranth from the cut surface of the shorter piece is the length of the piece. Morgan has shown that in branching stems a short piece of a branch or of a stem regenerates as does an isolated short piece, *i. e.*, the region of the tentacle anlagen is reduced and the rate of development of a hydranth is much slower than that of a hydranth from the cut end of a long piece of stem. Both of these factors may help to bring about the delay in the development of a hydranth from the oral end of the shorter piece in all of these experiments with branching stems in which there is a marked difference in the length of the stem and of the branch.

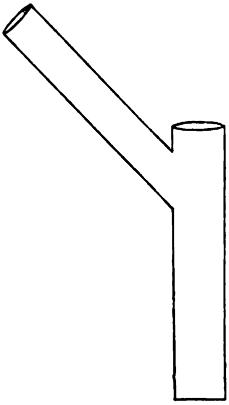


FIG. 4.

Series 2. — Seventeen branching stems from different colonies were cut so that the anterior portion of the stem above the place of union with the branch was only about 1 mm. in length, while the length of the branch varied in different cases from 8–25 mm. (Fig. 4). Two days after the operation, hydranths had developed at the cut ends of thirteen branches, and well developed tentacle anlagen were found at the oral ends of the other four branches. No hydranths were found at this time at the oral end of any of the stems, and tentacle anlagen were only faintly defined in some few cases. On the next day, hydranths had developed at the distal end of all of the branches, but only five stems bore hydranths at the oral end. In this set of experiments the development of a hydranth took place more rapidly from the cut end of the long branch than from the oral end of the main stem.

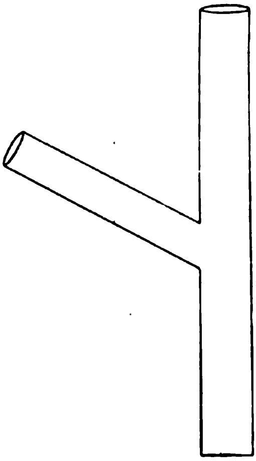


FIG. 5.

Series 3. — Twelve experiments were made in which the anterior end of the stem and the distal end of the branch were cut off so that the length of the branch and of the stem above the place of union was practically the same, varying in different cases from 8–20 mm. (Fig. 5). Two days after the operation, there were well developed hydranths on the ends of three branches and only tentacle anlagen on the oral ends of the corresponding stems. In two cases hydranths had developed on the end of the branch and also on the cut surface of the stem; while in the other cases only tentacle analgen were found, and they were equally well-developed on the branch and on the oral end of the stem. During the following two days, hydranths formed on all of the cut ends, sometimes the hydranth developed on the end of the stem before it did on the branch, and sometimes the hydranth appeared first on the branch. As a general result of this series of experiments it can be stated that hydranths develop at about the same rate when both the branch and the anterior portion of the stem are approximately the same length.

Series 4. — In the previous set of experiments both the branch and the anterior portion of the stem were of considerable length and both developed at about the same rate. In order to see if similar results would be obtained if the pieces were very short, fifteen experiments were made in which the branch and the stem were cut off about 1 mm. above their point of union (Fig. 6). When the hydroids were examined three days after the operation, hydranths were found on the oral end of the stem and not on the cut end of the branch in five cases; while in four hydroids, polyps had developed on the branch and not on the oral end of the stem; in the remaining six cases, hydranths were present at the distal end of both branch and stem.

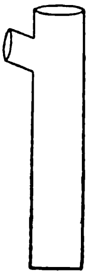


FIG. 6.

In those cases in which one or the other cut surface failed to develop a hydranth, the cœnosarc appeared to be entirely with drawn from this part. When these stems were examined under the microscope, the streaming of granules in the interior cavity was visible only in the proximal part of the main stem and in the part of the stem or branch that had regenerated. In the cases in which hydranths formed at both cut surfaces, the streaming of granules was found in all parts of the stem and also in the branch.

In this set of experiments, regeneration seemed to take place with equal rapidity from the cut oral surface of the branch and of the stem, as was the case in the experiments described under series 3. If the pieces above the place of union of the branch and stem are of approximately the same length, no matter how long or how short they may be, the influences for hydranth formation appear to be alike in both, and one piece has, seemingly, no effect on the other. Where the cœnosarc is withdrawn entirely from one part of the hydroid, as was noted in some few cases in the last set of experiments, no regeneration of the piece is possible.

In experimenting on *Tubularia crocea*, Morgan (9) found that if he cut off both the main stem and the branch a little above the place of union, the results varied considerably in different cases. In some instances he obtained the regeneration of a hydranth on the branch and not on the oral end of the stem; in other cases a polyp formed only at the oral end of the main stem; and in still other individuals regeneration took place from the cut surfaces of both branch and stem. It seems probable that the lack of uniformity in these results can be attributed to a difference in the relative lengths of the branch and of anterior portion of the stem above the point of insertion of the branch. It is, of course, impossible to cut the branch and stem at absolutely equal distances from the place of their union, and in those cases in which regeneration from one cut surface took place before it did from the other, there may have been just enough difference in the lengths of the pieces to bring about the earlier regeneration of a hydranth on the cut oral surface of the longer piece.

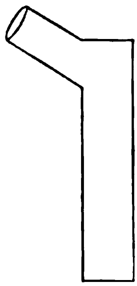


FIG. 7.

Series 5. — In twenty-eight cases the stem was cut off transversely just above the origin of the branch as shown in Fig. 7. The oral end of the branch was then removed leaving a piece, from 3 to 5 mm. in length, still attached to the stem. The object of these experiments was to see whether the regeneration of a hydranth at the oral end of the stem could be entirely prevented by this means. Two days after the operation, hydranths had developed at the cut end of the branch in nine of the hydroids and well-developed tentacle anlagen were present on the other branches; there was no indication of a regeneration at the oral end of any of the stems. The next day all of the branches bore hydranths, and in but one case had any regeneration taken place at the oral end of the stem. In this instance, the stem extended about 0. 5 mm. above the place of insertion of the branch and a considerable amount of red pigment had collected at its extreme oral end. In the course of forty-eight hours more a polyp formed on the oral end of this stem but no regeneration took place at the oral end of any of the other stems, although they were kept for over a week.

Series 6.—Sixteen experiments were made in which the branches were cut off very close to their origin on the stem. The oral end of the stem was then removed leaving a piece about 5 mm. in length above the origin of the branch, in order to see whether the formation of a hydranth at the oral end of the stem would prevent or merely delay the formation of a polyp at the place where the branch was removed. In all cases the wound in the side of the stem healed over very quickly and, although the hydroids were kept alive for a number of days, no regeneration of any kind took place at the point of injury.

Series 7. — In ten cases the entire branch was removed from the stem, but the old hydranth at the distal end of the stem was not cut off. The result was the same as in the previous set of experiments, as the cut surface was very soon covered over and no subsequent regeneration took place from it.

Series 8. — In sixteen cases where long pieces of stem bore from two to four branches, the anterior end of the stem and the apical end of each branch were removed by transverse cuts leaving the lengths of the branches approximately the same as that of the stem above the origin of the most anterior branch. The experiments were made to see if there is any difference in the relative rate of regeneration of the anterior branches and of the proximal ones. There was no uniformity whatever in the results of this set of experiments. In some cases a hydranth regenerated on a posterior branch before it did on the oral end of the main stem; and in other cases all of the branches produced hydranths at the same time that one developed at the oral end of the stem.

III. The Regeneration of Short Pieces of the Stem of Tubularia.

It was first noted by Bickford (1), and later confirmed by Driesch (2) and by Morgan, that small pieces of the stem of *Tubularia* about 1 mm. in length are capable of regenerating. In a recent paper, Hargitt (5) states that he was unable to obtain any regeneration from pieces of the stem of *Tubularia crocea* and of *Tubularia tenella* that were as much as 3–4 mm. in length. This result was probably due to the poor condition of the stems when the experiments were made. Small pieces of the stem of some other hydroids, do not appear to possess as great a power of regeneration as *Tubularia*, for Gast and Godlewski (4) have found that pieces of the stem of *Pennaria cavolinii* about 1 mm. in length never produce hydranths and pieces 2 mm. in length regenerate hydranths but rarely. Bickford's experiments on small pieces of the stem of *Tubularia tenella* show that, in this species, regenerative processes are not restricted to any special region of the stem, and also that such short pieces tend to form one complete hydranth rather than to produce double abnormal structures.

In experimenting on *Tubularia mesembryanthemum*, Driesch (2) found that of 82 short pieces of stem, 5 formed a single proboscis, 26 formed a double proboscis, and the remaining 51 pieces produced hydranths. These results agree with those of the earlier experiments of Bickford. In a later paper, Driesch (3) states that at the oral end of the stem one seldom gets a whole hydranth, but usually a single or a double proboscis; from the middle zone hydranths usually develop; while from the aboral end of the stem, these structures are rarely produced. This difference in the kind of regeneration from the various parts of the stem Driesch attributes to the situation of the small piece in the original individual and to the different distribution of the hydranth-forming pigment in the cœnosarc of the different parts. Since Morgan and also Stevens (11–12) have proven the fallacy of the hypothesis of "red formative stuff" in *Tubularia*, this portion of Driesch's explanation is, of course, no longer tenable.

Morgan (8–10) has made an extended series of experiments with small pieces of the stem of both *Tubularia mesembryanthemum* and of *Tubularia crocea.* He finds, as did Driesch, that pieces about 1 mm. in length from the region immediately behind the old hydranth usually die, even when longer than pieces from a more proximal region that regenerate. When this distal region does regenerate, it produces a greater number of single proboscides than of other forms, a result that might be expected as this part of the stem ordinarily goes into the proboscis of the new hydranth when a long piece of stem is regenerating.

In another set of experiments, Morgan cut pieces of the stem of *Tubularia mesembryanthemum* into a series of small pieces about 1 mm. in length in order to observe the behavior of consecutive pieces from one stem and to compare the results with those obtained from similar pieces cut from other stems. His tables do not show any very definite results although there seems to be a certain similarity in the behavior of pieces of the same stem, and the incomplete structures are found most frequently at the distal end of the stem.

At the suggestion of Professor Morgan, I repeated his experiments, using *Tubularia crocea,* in order to furnish more data from which definite conclusions could be drawn. In making the experiments the old hydranths were removed and the distal part of the stem was cut into consecutive pieces about 1 mm. in length. The pieces were than laid in rows on the bottom of flat dishes filled with fresh sea water. For the sake of brevity the following abbreviations are used in the tables given : hy. = complete hydranth without any stalk ; hy. + stalk = hydranth with a short stalk that has been formed by a withdrawal of the cœnosarc from the perisarc ; hy. + stem = hydranth with a stem attached to the perisarc ; pb. = single proboscis ; d. pb = double proboscis ; reprod. = reproductive organs. The results tabulated are from observations made three to four days after the operation.

TABLE V.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb. with no tentacles.	6	pb.
2	d. pb.	7	d. pb.
3	pb.	8	hy. + stem.
4	d. pb. + reprod.	9	d. pb.
5	pb.	10	d. pb.

TABLE VI.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	pb.	6	dead.
2	d. pb.	7	pb.
3	d. pb.	8	pb.
4	dead.	9	dead.
5	d. pb.	10	dead.

TABLE VII.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	d. pb.	6	d. pb.
2	pb.	7	d. pb. + reprod.
3	pb.	8	pb.
4	pb.	9	hy. + stem.
5	hy.	10	pb.

TABLE VIII.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	dead.	6	pb.
2	d. pb.	7	pb.
3	d. pb.	8	hy. + stalk.
4	pb.	9	pb.
5	pb. + reprod.	10	d. pb.

TABLE IX.

Number of Piece.	Kind of Structure Regenerated.	Number of Piece.	Kind of Structure Regenerated.
1	dead.	6	pb. + reprod.
2	pb.	7	pb.
3	pb.	8	hy. + stem.
4	hy. + stem.	9	pb.
5	pb.	10	dead.

TABLE X.

Number of Piece.	Kind of Structure Regenerated	Number of Piece.	Kind of Structure Regenerated.
1	pb.	6	pb.
2	d. pb.	7	hy. + stem.
3	d. pb.	8	pb.
4	d. pb. + reprod.	9	hy.
5	pb.	10	dead.

As was the case in the experiments made by Morgan, the results for corresponding pieces of different stems are far from uniform, and it is not possible to determine what kind of a structure will be produced by a small piece from a given region of the stem. It is evident that the power to form either complete or double structures is present throughout the stem, and just what conditions are necessary to produce certain structures have not, as yet, been fully determined. Morgan has suggested that possibly the factors in determining the kind of regeneration are (1) the smallness of the piece, (2) the differences in the region of the original stem from which the pieces came (this factor had been previously suggested by Driesch), (3) the age of the piece, as the younger the stem the more likely it would be to form incomplete structures.

According to this set of experiments short pieces, no matter from what part of the stem they are taken, are more liable to produce proboscides than to form hydranths. When the latter structures appear they are usually produced by the more proximal pieces of the stem, the distal end of the stem showing a great tendency to produce incomplete structures. These results are very similar to those obtained by Morgan on *Tubularia mesembryanthemum.*

In order to ascertain whether the double structures that are so often obtained in such experiments are produced because the small pieces of the stem are open at both ends and not because there is insufficient material in the piece to produce a complete hydranth, Morgan tied one end of a short piece with silk thread, and found that, under these conditions, double structures are never produced. Later he planted short pieces of stems in rows in sand so that one end was buried and the other freely surrounded by water. In two instances only was a double proboscis formed, in all other cases single structures, either incomplete or whole, more often the latter were produced.

In repeating these experiments of closing one end of a short piece of the stem of *Tubularia* in order to ascertain the effect on the kind of structure produced, the following method was used: Shallow, flat dishes were covered on the bottom with a layer of paraffine about one fourth of an inch in thickness, and then, with the blunt end of a large needle about the diameter of a tubularian stem, rows of holes were made in the paraffine about 0.5 mm. in depth. The dishes were filled with water and the long pieces of stems were put in them and cut into consecutive pieces about 1 mm. in length. One end of each piece was then inserted in a hole that was just large enough to receive and hold it upright. This method has the advantage that the inserted end of the piece of stem is in contact with the paraffine and cannot become free if the experiment is properly done. The results of this series of experiments are summarized in the following tables in which the abbreviations used are the same as those previously employed.

TABLE XI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb.	6	pb.
3	pb. + reprod.	7	dead.
4	dead.	8	pb.

TABLE XII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	hy.	7	pb.
4	dead.	8	dead.

TABLE XIII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	dead.	6	pb. + reprod.
3	d. pb.	7	pb.
4	dead.	8	pb.

TABLE XIV.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	hy.	5	dead.
2	pb.	6	dead.
3	pb.	7	pb.
4	pb.	8	dead.

TABLE XV.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	dead.
3	pb. + reprod.	7	dead.
4	pb.	8	dead.

TABLE XVI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	pb.	7	pb.
4	hy. + stem.	8	dead.

TABLE XVII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb. + reprod.	6	dead.
3	pb.	7	dead.
4	pb. + reprod.	8	dead.

TABLE XVIII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	dead.	5	dead.
2	pb.	6	hy. + stalk.
3	pb.	7	pb.
4	pb.	8	dead.

TABLE XIX.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb. + reprod.	6	dead.
3	hy. + stalk.	7	hy. + stem.
4	pb.	8	hy. + stem.

TABLE XX.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	pb.	6	pb.
3	hy. + stem.	7	pb.
4	dead.	8	dead.

TABLE XXI.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	dead.
2	pb. + reprod.	6	pb.
3	pb.	7	pb.
4	hy. + stem.	8	dead.

TABLE XXII.

Number of Piece.	Structure Produced.	Number of Piece.	Structure Produced.
1	pb.	5	pb.
2	dead.	6	hy. + stalk.
3	pb. + reprod.	7	pb.
4	pb.	8	dead.

The results of these experiments confirm those obtained by Morgan in every respect, as double structures were produced very rarely, only one being obtained in the entire series of experiments. There seemed to be no distinctive individual differences in the pieces of stem as regards the structures produced. In only one case (Table XIX.) were as many as three hydranths produced, while in the tables given by Morgan for *Tubularia mesembryanthemum*, whole series of pieces from the same stem produced hydranths.

If a comparison is made between the results shown in Tables XI. to XXII. and those shown in Tables V. to X., the most noticeable difference is that a very much greater number of double structures were produced when short pieces of the stem were lying on their sides during the process of regeneration. In both sets of experiments single proboscides were the structures most frequently produced, and very little individual difference could be detected in the stems regarding the kind of structure that they would tend to produce.

The development of small pieces of the stem of *Tubularia* standing on one end is considerably slower than that of similar pieces lying on one side. In the latter case, development takes place in about two days; while in the former case the various structures never appear under three days, and usually not under four or five days. Many pieces, usually those nearer the proximal end of the stem, die when the pieces stand on one end. This result may be due to the fact that the conditions under which regeneration takes place are not as favorable when the end of a small piece of stem is closed by contact with some foreign substance, as when the piece lies on one side and the cut ends are allowed to close in a normal manner.

Bryn Mawr College,
Bryn Mawr, Pa., March 23, 1904.

LITERATURE.

1. **Bickford, Elizabeth.**
'94. Notes on Regeneration and Heteromorphosis of Tubularian Hydroids. Journ. of Morph., Vol. IX.

2. **Driesch, Hans.**
'97. Studien über das Regulationsvermögen der Organismen, I. Von dem Regulativen Wachsthums- und Differenenzirungsfähigkeiten der Tubularia. Arch. Entwickelungsmech., Bd. V.

3. **Driesch, Hans.**
'99. Studien über das Regulationsvermögen der Organismen, II. Quantitative Regulationen bei der Reparation der Tubularia. Arch. Entwickelungsmech., Bd. IX.

4. **Gast, R., und Godlewski, E.**
'03. Die Regulatonserscheinungen bei Pennaria cavolinii. Arch. Entwickelungsmech., Bd. XVI.

5. **Hargitt, G. T.**
'03. Regeneration in Hydromedusæ. Arch. Entwickelungsmech., Bd. XVII.

6. **King, H. D.**
'03. Further Studies on Regeneration in Hydra viridis. Arch. Entwickelungsmech., Bd. XVI.

7. **Loeb, J.**
'92. Untersuchungen zur Physiologischen Morphologie der Thiere, II. Organbildung und Wachsthum. Wurzburg.

8. **Morgan, T. H.**
'01. Regeneration in Tubularia. Arch. Entwickelungsmech., Bd. XI.

9. **Morgan, T. H.**
'02. Further Experiments on the Regeneration of Tubularia. Arch. Entwickelungsmech., Bd. VIII.

10. **Morgan, T. H.**
'03. Some Factors in the Regeneration of Tubularia. Arch. Entwickelungsmech., Bd. XVI.

11. **Stevens, N. M.**
'01. Regeneration in Tubularia mesembryanthemum, I. Arch. Entwickelungsmech., Bd. XIII.

12 **Stevens, N. M.**
'02. Regeneration in Tubularia mesembryanthemum, II. Arch. Entwickelungsmech., Bd. XV.

The Regeneration of the First Leg of the Crayfish.

By

Margaret A. Reed.

With Plate XVII—XVIII and 3 figures in text.

Eingegangen am 1. Februar 1904.

Owing to the presence of a definite breaking-joint in the second podomere of the first pair of legs of the crayfish, and to the fact that no muscles cross the breaking joint, the question arises as to how the cells to form the new muscles of the regenerated leg arise, whether from the old muscles, which are uninjured, or from some other source.

Under the direction of Professor MORGAN, the study of this problem was carried on in the Biological Laboratory of Bryn Mawr College during the winters of 1902 and 1903.

Three sets of crayfish, varying in length from two to eight centimeters were used. They were found in the brooks about Bryn Mawr. The species was not determined. The crayfish were kept in glass dishes under running water and fed occasionally with earthworms. In all cases the first leg or cheliped was operated upon. If the distal end is injured at any level autotomy takes place at the breaking joint.

The walking legs may be thrown off by autotomy also, but not with as great ease as the cheliped. The walking leg must be held with the scissors or forceps after it is cut in order to cause autotomy to take place. The leg is thrown off, not at a special plane in the exoskeleton of the second podomere, but at the free joint between the second and the third podomeres.

Occasionally it is impossible to cause autotomy of a walking leg. This is particularily true if one leg has already been thrown off. In such a case a new leg regenerates from the cut-surface, although the regeneration is much slower than when it takes place from the breaking joint.

The crayfish were killed and preserved at varying intervals to obtain a series of stages showing the development of the new leg from the time the leg is thrown off until it is fully formed. The following series was preserved: — 0, 15 mins., 30 mins., 1 hr., 3 hrs., 7 hrs., 10 hrs., 12 hrs., 15 hrs., 18 hrs., 24 hrs., 32 hrs., 48 hrs., . . . 480 hrs., 492 hrs., 504 hrs.

Usually at the end of three weeks the new leg is plainly visible to the naked eye. It consists of the normal number of podomeres, with the chelae well formed, but it is much smaller than the normal leg. The size of the leg increases with each succeeding moult until it attains the size of the lost leg.

When the crayfishes were killed, the thorax was opened and placed in corosive acetic (corosive sublimate, 95 c. c. + glacial acetic, 5 c. c.), hardened, decalcified in nitric acid (HNO_3, 2 c. c. + H_2O, 98 c. c.; or 70% alcohol, 98 c. c. + HNO_3, 2 c. c.); embedded in hard paraffine, sectioned, and stained on the slide with DELAFIELD's Haematoxylin (differentiated with Orange G). This stains the nuclei a purplish blue, while the blood, chitin and protoplasm of the connective tissue cells are yellow.

The normal cheliped of a crayfish consists of six podomeres, each of which has an extensor and a flexor muscle. These muscles are attached to the exoskeleton of the distal podomere by a central tendon, — a plate-like ingrowth of the exoskeleton toward which all the muscular fasciculi converge. These muscles cross the joint and are inserted on the proximal podomere just back of the membrane of the joint.

The second podomere is peculiar in that it has two grooves around it, each lacking hairs and hair-pores in the exoskeleton along these lines. When the leg is injured distal to this place, it separates by a reflex action along the proximal groove. ANDREWS 1) calls this groove the breaking joint and describes it as, »a definite plane of discontinuity in the lamellar structure of the chitinous exoskeleton at the proximal groove«. Extending from the exoskeleton part of the plane of rupture there is a definite structure in the soft part of the leg, forming a double-walled membrane extending inwards from the epidermis to the central nerve and blood vessel. When the leg is

---

1) American Naturalist. Vol. XXIV. No. 278.

thrown off, the distal fold is cast off with the leg while the proximal fold remains attached to the stump.

After autotomy, the edge of the chitinous exoskeleton presents a smooth surface, slightly convex and sharply cut off, entirely different from the ragged edge presented when the leg is broken at any other level. Almost no bleeding takes place, when the leg is thrown off at the breaking joint. One or two drops of blood are shed, which form a clot in the opening through which the nerve and blood vessel passed. The membrane, with the opening filled with a clot of blood, is plainly visible after the leg has been thrown off, in the crab, lobster, or large crayfish.

In the lobster and the hermit crab the opening through the membrane for the nerve and the blood vessel is in about the center of the exposed surface when the leg is thrown off, but in the crayfish the opening is not in the center of the membrane but to one side. The membrane does not extend over to this side, or if it does, it is so small a fold as to give the appearance of none.

The breaking-joint divides the second podomere into two parts, a proximal and a distal part, each of which possesses a flexor and an extensor muscle. The flexor muscle of the proximal part consists of four branches, which have their tendons attached distally on the same trunk, formed by a chitinous ingrowth of the exoskeleton slightly below the level of the breaking joint (Fig. 3 *a*), while their proximal insertions are separate. The largest bundle of fibers, belonging to this muscle, is inserted along the ventral edge of the posterior wall of the first thoracic segment. Another bundle is attached deeper to the lateral dorsal wall of the same segment, while the other two bundles are inserted on the wall of the first podomere of the leg. The flexor muscle is larger than the extensor muscle, and consists of two branches each with its own attachment distally and proximally although near together. One branch has its tendon formed as an ingrowth from the ridge of the exoskeleton just beneath the breaking joint and dorsal to the hinge of the joint between the first and the second podomere, Fig. 3 *b*. It passes as a thin bundle of fibers to the dorsal lateral wall of the first thoracic segment where it is inserted. The other bundle is thick and short. It has its tendon formed as an ingrowth from the exoskeleton near that of the first bundle (Fig. 3 *c*), and is inserted on the wall of the first podomere of the leg. The nerve and blood vessel lie in the space between the extensor and the flexor muscle.

The distal portion of the second podomere also possesses an extensor and a flexor muscle although small in comparison with the other muscles of the leg. The tendon of the flexor muscle is formed as an outgrowth of two branches from the ventral wall of the third podomere, Fig. 3 *e*. The muscle bundles are attached along the edge of the breaking-joint at the dorsal side of the second podomere. The tendon of the extensor muscle is an outgrowth of the edge of the third podomere near that of the flexor muscle, but nearer the dorsal wall (Fig. 3 *d*). The muscle bundles partly cross those of the flexor and are inserted along the edge of the breaking joint at the ventral side of the podomere.

As far as I have been able to make out, autotomy takes place as follows. A contraction of the extensor muscle of the proximal portion of the second podomere draws the leg sharply back against the carapace of the thorax. Then, probably, by a contraction of the flexor muscle of the distal portion of the second podomere, the leg is separated along the breaking point.

That the extensor muscle is the muscle chiefly involved has been shown by MORGAN in his experiments on hermit crabs, where he found that when this muscle was cut, autotomy did not take place at the breaking joint. In my experiments on both hermit crabs and crayfish, I found that cutting this muscle would prevent autotomy. FREDERIQUE[1]) has shown that autotomy is not due to a weakness at the breaking joint, but to a reflex action, and that it may be brought about by a stimulation of the thoracic ganglion as well as by a stimulation of the nerve of the leg itself. MORGAN[2]) has shown that section of the nerve at the base of the leg, or proximal to the breaking joint will prevent autotomy.

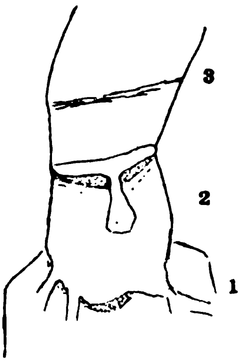
It has usually been taken for granted that the breaking joint is merely a modified joint. The double fold of membrane and the plane of discontinuity in the exoskeleton have something of the structure of a joint between two podomeres. The fact that the first pair of legs or the chelipeds have only six podomeres, while the walking legs have seven, and also the fact that the walking legs may be thrown off, although with difficulty, at the free joint between the second and the third podomeres, seemed to add to the evidence that the breaking-joint had once been a true joint, which is no longer used as a joint, but has become differentiated for the purpose of casting off the leg when the leg is injured.

---

[1]) »Travaux du Labaratoire«. I—II. 1887/8.

[2]) Anat. Anz. XVII. 1900.

Fig. 1.



Comparing the different thoracic legs of a lobster, I find that the fusion of a joint has taken place not between the second and the third podomeres of the leg but between the third and fourth podomeres. Figs. 1, 2 and 3 illustrate this point. Fig. 1 is one of the third pair of walking legs (composed of seven podomeres). The third podomere, 3, is small and the joint between the third and the fourth podomeres does not move easily. Fig. 2 is one of the second pair of walking legs and here the joint between the third and the fourth podomeres is marked, but it is not a real joint and no movement occurs at this place. Fig. 3 is the cheliped, and here we have no sign of the joint between, the third and fourth podomere which have fused, but we do have the breaking joint appearing in the second podomere. Here the arrangement of the muscles is not that of the joint between the third and the fourth podomeres, which have become fused. It seems therefore erroneous to state that the breaking joint corresponds to the lost joint, for in the lobster, at any rate, this is not the case.

Fig. 2.

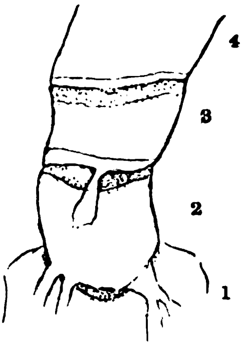
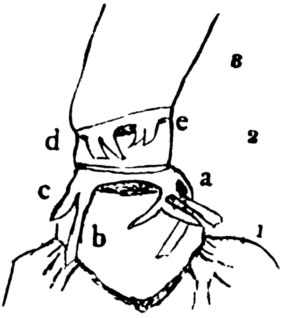


Fig. 3.



The internal structure of the normal leg has been described above. In the crayfish, sections through the breaking joint do not show the membrane for the breaking joint as stretched completely across the stump, but as lacking on the dorsal side or very slightly invaginated and not extending inwards to the central nerve. This is not the case in the lobster or hermit crab. Fig. 1, Pl. XVII shows a section through the breaking-joint of a normal leg. The plane of discontinuity in the chitin is at right angles to the long axis of the leg, and extending in from this is the double fold of cells (*E*), which form the membrane of the breaking joint. The nerve (*n*) passes nearer to the dorsal wall and on this side the definite double layer of cells is not shown although the plane of discontinuity (*sd*) in the exoskeleton is present. The space between the nerve and the wall of the exoskeleton is filled with connective tissue cells (*st*). The large blood vessel, which is not shown in this figure, lies close beside the nerve (*n*).

Almost immediately after autotomy has taken place, the ectoderm cells of the membrane are found stretched across the opening through which the nerve and blood vessel passed. Fig. 2, shows a section through a stump from which the leg has been thrown off for fifteen minutes. The double layer of flat cells (*E*), which form the proximal fold of the membrane have sent out a single layer (*e*) over the opening from which the torn end of the nerve has slightly withdrawn. Small round cells, with deeply stained nuclei, appear at the broken end of the blood vessel and scattered through the connective tissue. These blood cells (*b*, *c*) form a plug at the opening through the membrane, and collect in a thick layer just beneath the ectoderm cells (*E*), which form the proximal layer of the membrane of the breaking joint, as may be seen in Fig. 3. The whole stump is filled with blood, which appears as fine granules, stained yellow, and fills up the spaces between the cells. The ectoderm cells, which line the exoskeleton of the leg are seen as a columnar layer (*E'*), just beneath the chitin and in some sections slightly drawn away from the exoskeleton. The nuclei of these cells are larger and more granular than those of the connective tissue cells or the blood cells. These cells remain at rest for some days while the blood cells continue to fill up the interior of the stump. After a time these blood cells arrange themselves in horizontal lines crowded close under the ectoderm cells of the membrane, Fig. 3. In a few hours from the time the leg is thrown off, the membrane takes on the appearance of dead tissue. This membrane, together with the blood cells beneath, serves as a protection for the broken end of the stump, but they take no farther part in the regeneration of the new leg. The blood cells soon degenerate as may be seen in Fig. 6 and most of them are thrown away with the membrane, when this is later cast off.

The processes, which have gone on up to this time have been a sort of preparation for the regenerative processes, which begin about five days after the leg has been thrown off. At this time the columnar epithelium (*E'*) lining the exoskeleton begins to push forward through the blood cells to form a single layer over the broken surface, Fig. 4. No mitotic figures are found until after the ectoderm has formed a layer over the exposed surface. The cells push out from all sides and meet near the center, thus forming a complete, one-celled layer over the broken end. Later these ectoderm cells secret chitin over their surface, and bulge outwards through the protective covering formed by the old membrane and blood cells.

Fig. 5 shows a section through the proximal part of the second podomere where the new ectoderm cells (*E'*) have formed a layer over the broken end. The old ectoderm cells of the membrane (*E*) of the breaking joint together with the blood cells beneath, still form a covering over the exposed surface. The nuclei of the blood cells, Fig. 6, within the stump are breaking up and disappearing, but at this time there are still a great many of them scattered through the connective tissue.

Shortly after the ectoderm cells have formed a layer across the broken end, the cells in the central part of this layer begin to proliferate forming a thickened disc, distal to the broken end of the nerve. These cells divide by mitosis, and dividing cells are seen scattered through the proliferating part (Fig. 7). Many of these cells, as they are proliferated, push their way between the cells of the broken end of the nerve. Fig. 8 shows the ectoderm thickened with some of the cells pushing into the interior. A few blood cells still remain scattered through the tissue, but at this time the greater number of them have disappeared.

As the ectoderm proliferates, the new part begins to push out and soon breaks through the old membrane. A secretion of chitin is formed over the exposed surface, and this secretion continues to become thicker as the new part becomes older. In some crayfish this projection of the ectoderm cells occupies the greater part of what was the exposed surface, while in others it simply extends out from the central part. Fig. 9 shows a growing tip with the ectoderm cells everywhere proliferating and pushing into the interior. Mitotic figures (*FM*) are seen among the cells, which have been pushed in, as well as among those, which are still proliferating. As the cells push in they elongated, and many join the cells of the nerve. These later differentiate, the nuclei elongating and taking the yellow stain of the nerve-sheath cells. In Fig. 10, the pushing in of the ectoderm (E') is plainly shown, also two mitotic spindles (S) are to be seen among the proliferating cells. The ectoderm cells are forming the new nerve by adding themselves to the end of the old nerve.

As the new part of the leg grows out, it does not remain straight (Fig. 11) but folds in places, where later the joints between the podomeres form.

This folding in for the joints is brought about by the pushing in of the ectoderm cells in certain places. These cells continue to secret chitin so that a thin layer of chitin surrounded by ectoderm cells is formed extending into the interior. This pushing in takes place all the way around at the same time, as may be seen in Fig. 12, which is a section cut near the surface. This figure shows the beginning of this folding in. The cells which are differentiating to form the nerve are shown at N. Masses of ectoderm cells (mc), collected beneath the invagination of the ectoderm cells, will later differentiate to form the muscles. Many mitotic figures are seen, and the cells are proliferating throughout the growing end. All the cells present at this time have the appearance of ectoderm cells, except those, which are forming the nerve. These are elongated and stain a deeper yellow than the other cells.

After the pushing in has gone on for some time, a split appears along the line of chitin extending inwards from the outer surface. This split opens out and forms the fold for the joint. Although the split continues only for a short distance in the chitin, the ectoderm cells continue to grow inwards, and finally form the tendon for the muscle (Fig. 16).

The masses of ectoderm cells, which remain in the fold formed by the ingrowth of the ectoderm increase in number by the division of the cells already present, as well as by the addition of cells from the proliferating ectoderm of the exoskeleton, Fig. 13.

The ectoderm cells with their layer of chitin push in among these cells so that the tendon for the muscle lies surrounded by this mass of cells. After a short time these cells begin to elongate and to differentiate into muscle tissue. Fig. 14 shows such a stage in the development. The ectoderm cells of the exoskeleton have pushed in to form the tendon of the muscle, while the ectoderm cells of the inside have elongated and arranged themselves in lines extending from the ectoderm of the tendon to the ectoderm of the exoskeleton.

These cells continue to differentiate into muscle cells to form the fasiculi, which are attached along the ectoderm of the tendon and inserted among the ectoderm cells of the exoskeleton, Fig. 15.

The articulations between the podomeres do not appear until after the muscles are formed. Shortly after the muscles are formed, the exoskeleton, distal to the ingrowths for the tendons of the muscles, forms the hinge-joint with the membrane between secreted by the ectoderm cells, which were proliferated from the surface ectoderm.

I have compared the regeneration of the leg of the crayfish with that of the hermit crab (thrown off by autotomy at the breaking joint). Here almost the same development takes place. Figs. 16 and 17 show sections through the leg of the hermit crab at about the same stages in the regeneration as those represented in the crayfish by Figs. 7 and 9. The breaking joint has the same structure, except that the membrane is continued on the dorsal side, and the opening for the nerve and blood vessel is nearer the center of the membrane. The proliferation of ectoderm to produce the cells that will give rise to the new muscles takes place in the hermit crab in the same way as in the crayfish.

In closing I wish to mention a result obtained in the hermit crab by splitting the stump of the leg lengthwise after autotomy had taken place. In several cases after splitting the stump, two new legs appeared in a short while. Sections through this region showed that the nerve was split, one branch going to each leg. It is probable that a new leg developed at each end of the split nerve, since in all other cases where only one leg regenerated, the nerve shows no sign of any injury. In these cases it is probable that the nerve was not cut.

Summary.

When the first leg of the crayfish is thrown off at the breaking joint, no muscles are injured, and the muscles for the new leg are formed from cells proliferated by the ectoderm. Although the nerve is torn when autotomy takes place, the cells to form the new nerve (or at least the new nerve sheath), are also differentiated from cells proliferated by the ectoderm. In the hermit crab also the muscles of the new leg are formed by ectodermal cells.

If, after the leg of the hermit crab has been thrown off at the breaking joint, the base is split lengthwise so that the nerve is divided

or cut in two, there often appear two new legs, one connected with each end of the old nerve.

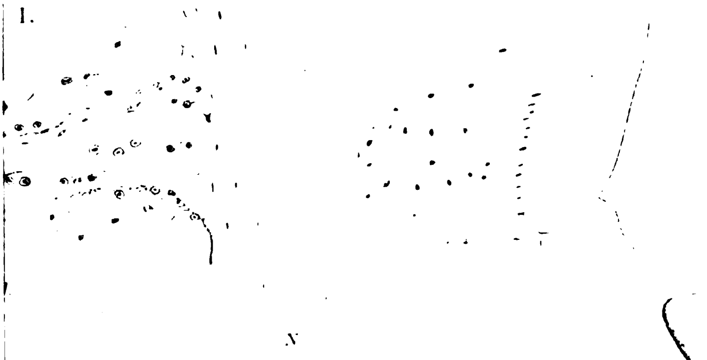
Bryn Mawr College, January 19, 1904.

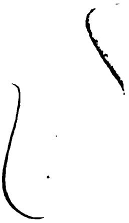
Zusammenfassung.

Wird das erste Bein des Flußkrebses durch Abbrechen des Gelenkes von diesem an entfernt, so wird kein Muskel verletzt und die Muskeln für das neue Bein werden durch Zellproliferation seitens des Ektoderms gebildet. Obgleich der Nerv bei stattfindender Autotomie (freiwilliger Abschnürung des Gliedes) zerrissen wird, so werden die Bildungszellen für den neuen Nerven (oder zum mindesten für die neue Nervenscheide) gleichfalls von sich differenzierenden durch Proliferation entstandenen Ektodermzellen gebildet. Beim Einsiedlerkrebs werden die Muskeln des neuen Beins gleichfalls durch Ektodermzellen gebildet.

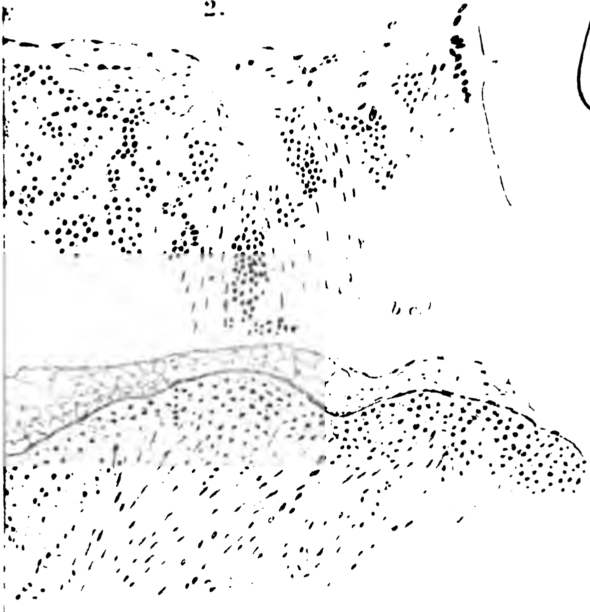
Wenn beim Einsiedlerkrebs das Bein durch Abbrechen des Gelenkes entfernt wurde, so ist die Basis der Länge nach gespalten, so daß der Nerv geteilt, der Länge nach getrennt wird. Dann erscheinen oft zwei neue Beine, jedes mit einem Stumpfteil des alten Nerven in Zusammenhang stehend.

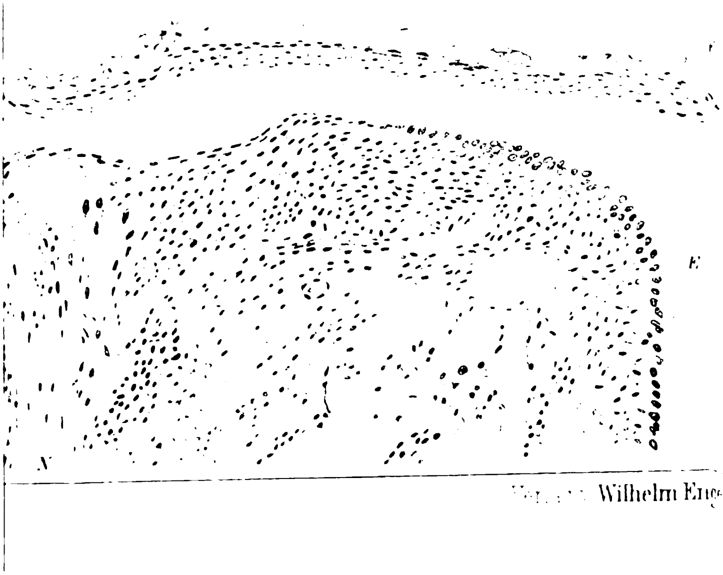
1.





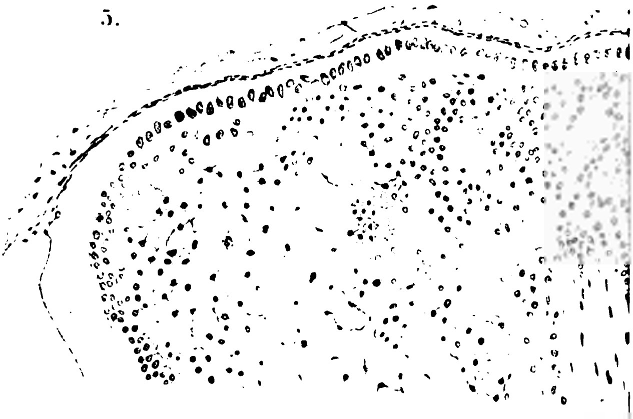
2.



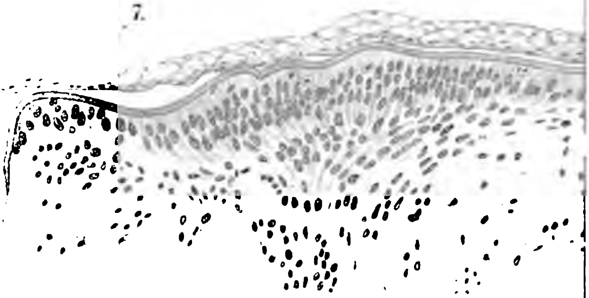


Wilhelm Enge

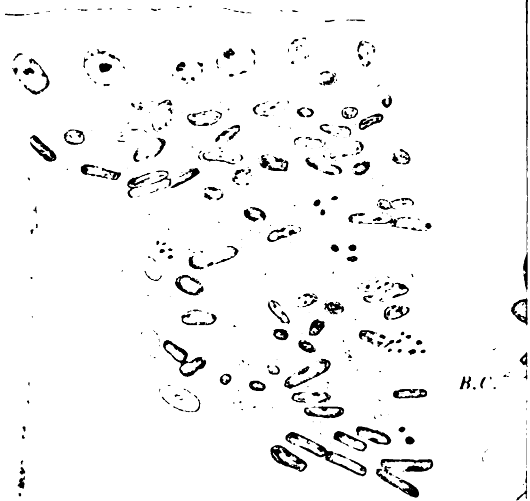
5.



7.

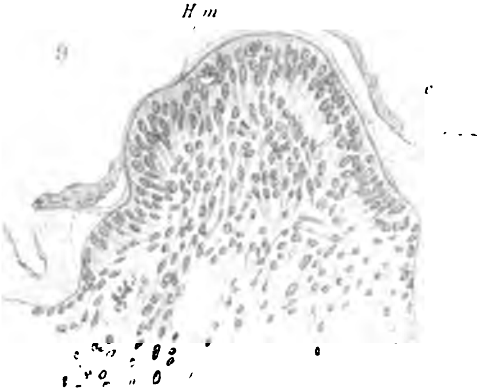


6.

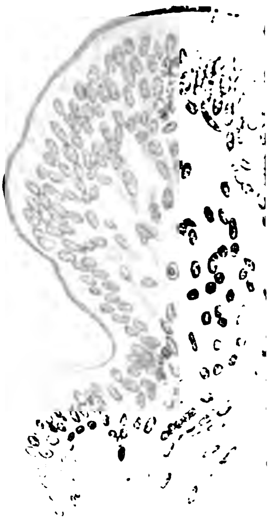


gelmann in Leipzig

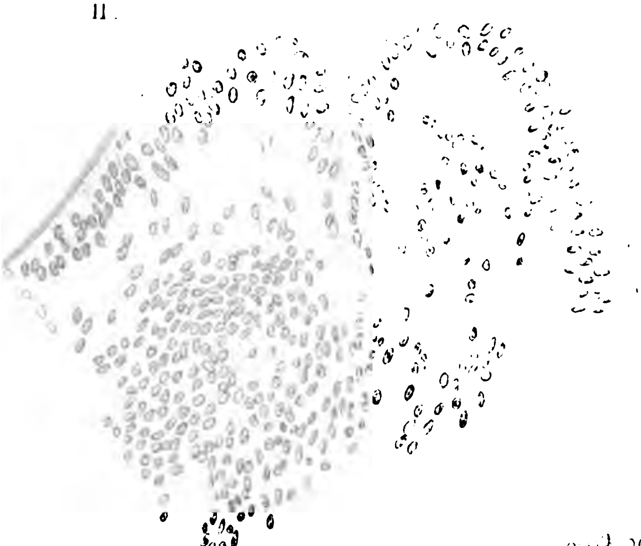
9.



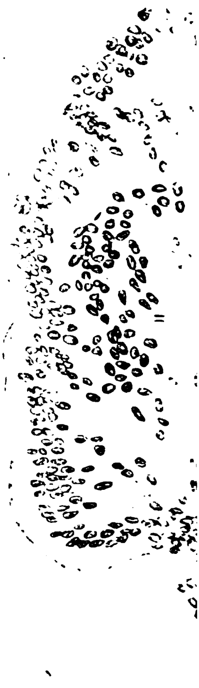
10.



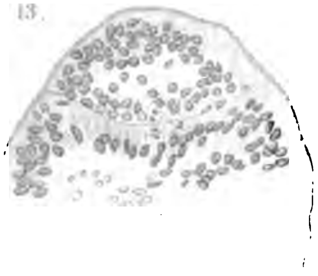
11.



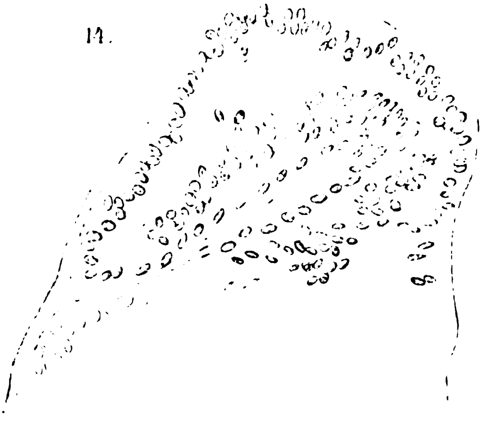
12.



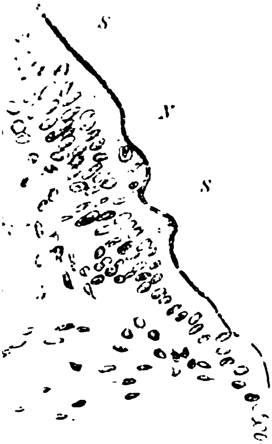
13.

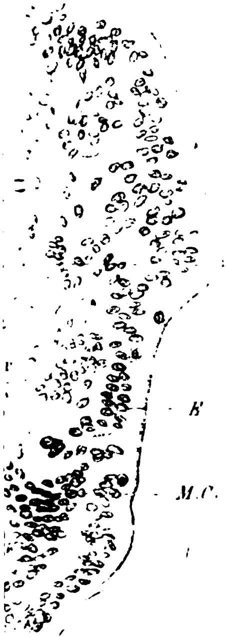


14.

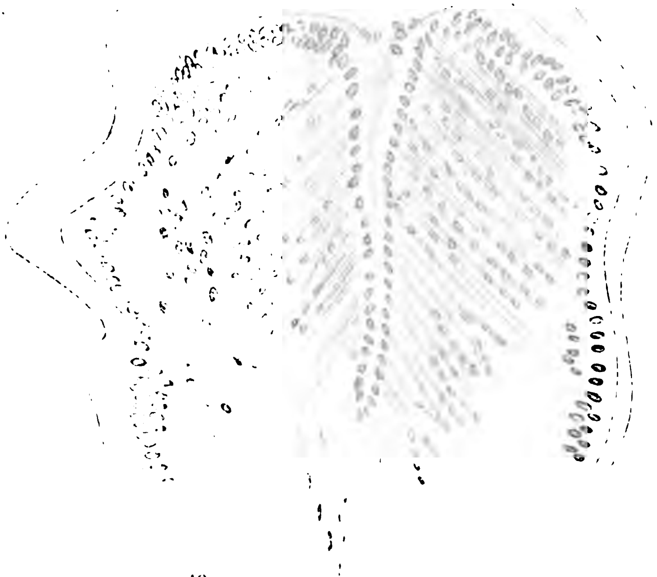


Wilhelm E

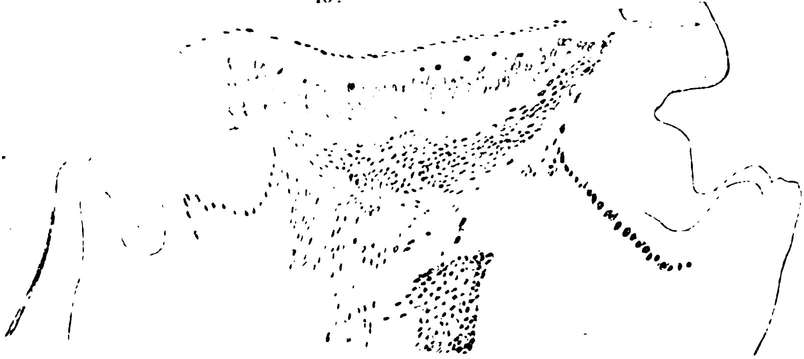




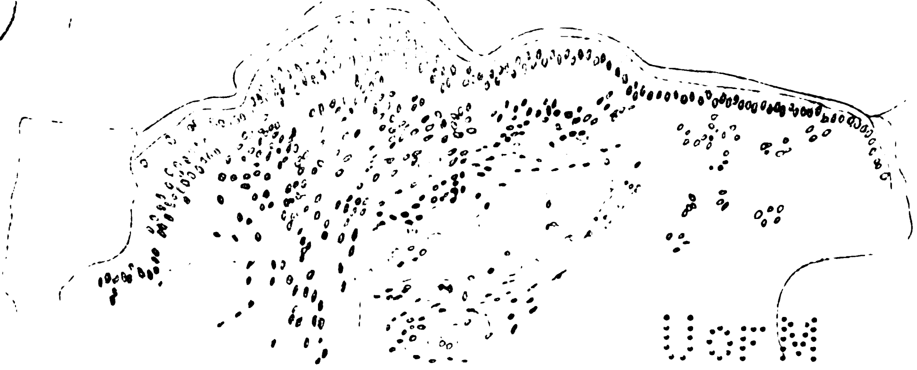
15.



16.



17.



UofM

gelmann [illegible]

Lith. Anst. v. [illegible]

[Reprinted from BIOLOGICAL BULLETIN, Vol. VII., No. 3, August, 1904.]

CLOSURE OF LONGITUDINALLY SPLIT TUBULARIAN STEMS.

ALICE M. BORING.

My work was carried on under the direction of Professor T. H. Morgan and Dr. N. M. Stevens, for whose suggestions and criticism I am deeply indebted.

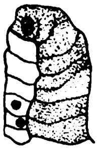
The rapid closure of the cut edges of pieces of the stem of *Tubularia* which have been split longitudinally, has been studied by Morgan and by Godlewski. The latter has published the results of his observations in two recent papers. Godlewski worked with *Tubularia mesembryanthemum*, found at Naples, while my own work has been on *Tubularia crocea*, from Wood's Hole, Mass. The method of closing in this form appears to differ in a number of points from that of *Tubularia mesembryanthemum* described by Godlewski. Material that had been collected by Professor Morgan during the summer was sectioned and studied. The conclusions drawn from this work were then verified on fresh material, of which sections also were made. The study of the living material, however, is not very satisfactory, as it is possible to see so little of what is going on. The circulation of the fluid inside of the open tubes and the gradual stretching across of the membrane, are all that can be seen clearly.

The splitting was done with a pair of fine scissors. The material was killed and fixed in corrosive acetic. Somewhere in the process of preserving, probably in the alcohols, the cœnosarc shrinks and loosens from the perisarc, which keeps its former size and shape. This makes it difficult to study their relation to each other in the closing process. The tissues of the cœnosarc are well preserved by the corrosive acetic solution and can be studied easily. The sections were stained with Delafield's hæmatoxylin, followed by picric alcohol, according to the method of Stevens ('01). The picric alcohol stains the granules in the endoderm cells bright yellow, and thus makes the difference between ectoderm and endoderm cells distinct.

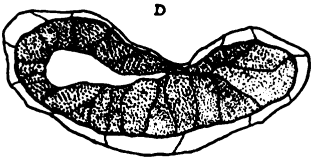
METHOD OF CLOSURE.

As the result of the splitting, the stems were divided into part-cylinders of various sizes, some almost complete, others with a mere strand of cœnosarc; while in some, an oblique split produced pieces nearly complete at one end and very small at the other. The method of closure seems to be independent of the size of the piece, the same general process being followed in all pieces. The cut leaves a free edge of both ectoderm and endoderm. In sections of pieces which had been killed five, ten or fifteen minutes after the splitting, the endoderm cells had rounded up over the ectoderm, probably as a result of disturbing the osmotic pressures (Fig. A). The first step towards closure of the tube is the pushing of the ectoderm over the endoderm (Fig. B). Whether this is accompanied by any activity on the part of the endoderm cannot be stated definitely, but it seems probable from some sections and from what occurs in later stages, that the endoderm cells change their shape, their outer ends next the ectoderm becoming broader and the inner ends becoming pointed,

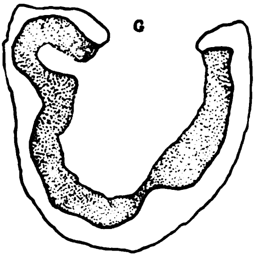
A



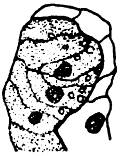
D



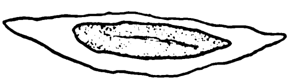
G



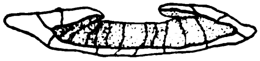
B



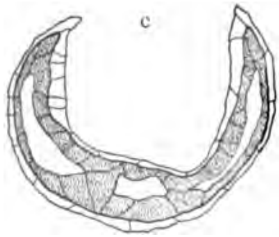
E



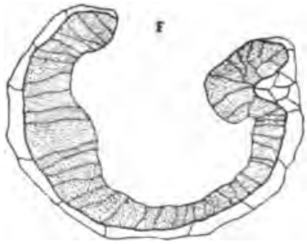
H



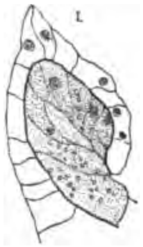
C



F



I



the cells thus taking on a triangular rather than a rectangular shape in section. This is shown in Fig. D. Some sections, however, do not show this at all, and we must conclude, that in some cases the endoderm lags a little behind the ectoderm.

During the subsequent process of closure, the ectoderm and the endoderm stretch across from both sides till they meet and form a two-layered membrane which closes in the digestive tract again. In this process of closing the two layers keep nearly even, but the ectoderm sometimes is a little farther advanced than the endoderm and curves around over its free end. The closing membrane does not arch outwards so as to complete the cylindrical form, nor does it grow straight across, which would be the shortest distance between the sides, but it is nearly always concave (Figs. C and D). In pieces which are much less than a half-cylinder, there is little chance for the membrane to bend in, and it lies close to the old wall (Fig. E). Also in cases where there is a large endodermal ridge, the bending in is either prevented or lessened.

The position of the endodermal ridges and their relation to the closure of the stem must now be considered. Godlewski has not taken these ridges into account, although one or two of his figures seem to indicate their presence. These ridges are found throughout the stem and cannot be ignored. Their presence causes some variations in the beginning of the process of closing and introduces certain conditions which might be easily misinterpreted. If the cut extends along the side of a ridge, or through it, the appearance in section of such a piece is very different from a section in which the ridge is not near the cut edge. There is a thick mass of cells at the edge, appearing somewhat like the beginning of a closing membrane (Fig. F). This mass is chiefly endoderm cells which contain many yellow-stained granules. If only a little of the ridge has been cut off, it is easily identified as a ridge by the thickening of ectoderm outside of it containing numerous dark stained nuclei; otherwise, identification is possible by tracing back through sections to a place where the cut was less deep and the ridge still intact. The ectoderm stretches over the cut endoderm until the two layers are even and then the two stretch across together and meet to form a complete membrane. If the cut edge extends just above a ridge, when the membrane begins to stretch across, a cavity is left between the closing membrane and the ridge, which in some cases looks like a canal within the edge of the closing membrane, such as Godlewski describes (Fig. G). It may be that his canals are formed in this way and his figures might be so interpreted. In the sections of *Tubularia crocea*, I have found no canals formed by the disintegration of endodermal cells in the edge of the closing membrane. In the living material, no circulation has been observed within the edges of the closing membrane indicating the presence of canals, yet the circulation in the bottom of the open cylinder was easily seen, so that the circulation is not something peculiar to the canals of Godlewski.

The pieces may be cut off in such a way that a ridge is left in the middle of the half-cylinders. Sometimes it appears as though the growing edge of one side joined such a ridge, but this appearance was seen in but a few consecutive sections, showing that it would not have interfered with natural closing, in further development. If the piece is rather small, or the ridge large, the closing membrane is prevented from bending in as much as usual. But this middle ridge makes still greater complications in small pieces, as here it may fill up the cavity formed by the closing membrane, and cause an appearance of solidity.

In many of the smaller pieces, the sections, at first sight, look solid as though the endoderm cells had remained inactive, and lay in the same position that they were in when the piece was cut. It looks as though the ectoderm has grown over this solid mass, thus making a solid core of endoderm with a thin covering of ectoderm as Godlewski describes. But it is natural to expect that if the endoderm cells lag only a little behind the ectoderm cells in the closure of a large piece of a tubularian stem, they would do the same in a small piece of the same stem. If the work had been entirely with large and small pieces coming from different stems or different portions of the same stem, this expectation might not be justifiable, but the pieces used were obliquely split, large at one end and small at the other, the conditions for both ends thus being nearly alike. A contraction, if the closing is due to this, would be along the entire free edge regardless of the size of the piece or the part of the tube, and if the endoderm and ectoderm both take part at one end, it is reasonable to expect that they would along the entire edge. With this idea in mind, the apparently solid closures were studied in order to see if there were not two layers of endoderm. The pieces were traced back through the series of sections to the place where less had been cut off in order to locate the ridges and in many pieces, a ridge was found included in the small piece. In some very small pieces, not including any ridge, it was found that the edges, both ectoderm and endoderm, had met to enclose a small but distinct cavity. Fig. H shows the section just before the edges meet. In other small pieces with no ridges the higher powers of the microscope showed two distinct layers of endoderm, indicating that the closure had been the same, but the piece was so flat and the angle turned by the closing membrane was so sharp that the cavity between the old and new walls was flattened out into a mere crack and the endoderm of the closing membrane practically touched the endoderm of the old wall (Fig. E). The presence of a ridge would cause the obliteration of the cavity in a piece otherwise large enough to leave a distinct cavity in closing. Fig. D shows this on the right side. The ridge is further toward the right, and consequently that side has no cavity. This section shows a circular arrangement of the endoderm cells at the point of turning, indicating a compression at their inner ends that would justify the statement that the layer of endoderm has been drawn around with the ectoderm.

In apparent contradiction to the above statement that only small pieces, or pieces including a ridge, close in solid, obscuring the cavity, a few sections complying with neither of these conditions show the ectoderm beginning to grow over the endoderm and leaving no cavity. But here, as in the small pieces there are two layers of endoderm in all cases where the ectoderm has overgrown, showing that the endoderm is following the ectoderm closely, and that a two-layered plate is really being formed even if obscured by the pressure of the two walls together (Fig. I). What the further development of such pieces would be, has not been definitely determined, but it is not likely that they close in to make a solid piece, for among the completely closed solid pieces, there were none so large, unless the stem was thicker, or a ridge intervened.

Whatever the cause of closure may be, whether some kind of contraction or not, the fact can clearly be seen from the study of sections that cell-division has little or no part in the formation of the closing membrane; the new tube has practically the same number of cells as the open, part-cylinder. Mitotic figures are occasionally found, but not more than might have occurred in a normal tubularian, and in any case they were not frequent enough to indicate that mitosis is a factor in forming the closing membrane.

BRYN MAWR,
May, 1904.

[Reprinted from BIOLOGICAL BULLETIN, Vol. VII., No. 3, August, 1904.]

REGENERATION IN THE PLANARIAN PHAGOCATA GRACILIS.

T. H. MORGAN AND ALICE E. SCHIEDT.

Phagocata gracilis differs from other planarians in having a number of pharynges in a common pharyngeal chamber. There is a large median anterior pharynx, and 12 or 14 lateral pharynges arranged along the sides of the chamber. The object of the following study was to determine the sequence in the regeneration of the pharynges in pieces from different regions of the body. The particular point that we had in view in beginning the work was to examine the origin of the new pharynges in pieces cut posterior to the region of the old pharynges. It has been shown for other planarians that pieces from this region of the body produce a new pharynx at or near the anterior end, and the question arose as to what would occur in a form like *Phagocata* having several pharynges. Would the median pharynx first appear and new ones be added behind this one? This would indicate that a growing region was also present *behind* the first pharynx, while in other planarians the growing region in pieces of this kind appears to be mainly anterior to the new pharynx. It seemed not improbable that a single anterior pharynx alone might develop and produce a mono-pharyngeal worm! Other similar questions arose in connection with the regeneration of new lateral pharynges in more anterior pieces.

The regeneration of the pharynges, in cross-pieces from the following regions of the body, was studied:

A. Worm cut transversely into five pieces.

1. Head-piece without any pharynges.
2. Median piece with old median anterior pharynx.
3. Middle piece with several lateral pharynges.
4. Posterior piece with posterior pair or pairs of lateral pharynges.
5. Tail piece without any pharynges.

B. Worm cut transversely into ten pieces.

C. Worm cut transversely into three regions: (i.) head, (ii.) region of pharynges, (iii.) tail piece. These three pieces were again cut transversely into as small pieces as possible.

An examination of the position of the new pharynges in the different pieces showed that in head-pieces the development occurred in the posterior part of the piece. In middle pieces new pharynges were regenerated at both ends; and in tail-pieces a new median pharynx appeared in the anterior part and new lateral ones were later added behind.

The first series (A) of preparations (sections) were of worms cut into five pieces and killed ten days later.

Five head-pieces: (i.) has a large central pharynx, four lateral pharynges, three on the left, one on the right.

(ii.) A central and three lateral pharynges are formed.

(iii.) A central and two lateral pharynges are regenerated.

(iv.) Four pharynges are formed; the largest is probably the median pharynx, but this is not certain because the piece had a peculiar shape, due to unequal cutting.

(v.) A central and four lateral pharynges are formed.

Five median pieces with the old central pharynx present.

(i.) The old central pharynx appears and five new lateral ones.

(ii.) An old central pharynx and, owing to imperfect cutting, old lateral ones are also found; three new lateral pharynges have regenerated.

(iii.) An old central pharynx and many new lateral ones are present.

(iv.) No old pharynx is found in this piece; a new central and three new lateral ones are formed.

(v.) An old central pharynx and possibly two or three lateral ones are left. There are several new lateral ones.

Five middle pieces. (i.) shows a new central pharynx and lateral ones anterior and posterior — the old lateral ones occupying the middle of the piece.

(ii.), (iii.), (iv.), and (v.) show a similar regeneration.

Of the five posterior pieces (i.), (iii.), and (v.) were cut too far back, and therefore only new pharynges are found. There are present a central and several lateral ones.

(ii.) and (iv.) have old lateral pharynges, and a new central pharynx and new lateral ones both anterior and posterior.

All the tail pieces show a new central pharynx and from two to five new lateral pharynges.

The second set of worms were cut into five similar pieces and killed at the same time as the first set.

Of the three head pieces (i.) and (iii.) show a new central and four new lateral pharynges.

(ii.) shows a central and three new lateral pharynges.

The median pieces all have a new central pharynx growing probably from the base of the old one. They all have several new lateral pharynges ; and worms (ii.) and (iii.) have two lateral old pharynges.

Each of the middle pieces shows many old lateral pharynges. (i.) shows one new lateral pharynx in the posterior end ; (ii.) shows two new anterior and one new posterior pharynx ; (iii.) and (iv.) show a new central pharynx and lateral ones anterior and posterior.

The posterior pieces (ii.) and (iii.) show a new central and new lateral pharynx in the anterior part, old lateral ones in the middle, and new lateral ones posteriorly.[1] (ii.) shows only a new central pharynx ; the rest are old. Of the tail-pieces, (i.) has a new central and five new lateral pharynges. In (ii.) no new pharynges have regenerated.

In (iii.) a new central and two lateral pharynges have developed.

The third set, cut into five pieces, were killed twelve days after the worms were cut.

The three head-pieces show a new central and four new lateral pharynges.

Of the median pieces (i.) and (iii.) show an old central and three new lateral pharynges. (ii.) has regenerated two new lateral ones posteriorly ; the four others, including the central one, are probably old ones.

The new central and several old lateral pharynges are present in the middle pieces ; new lateral ones are developed at both ends of the pieces.

Of the posterior pieces (i.) has one old and two new pharynges ; the central one is not yet regenerated.

---

[1] In this case it is probable that the cut removed some of the old posterior pharynges.

(ii.) has no old pharynges; but two new ones, one of which is the central pharynx. (iii.) has no old lateral pharynges in the posterior part, three new anterior ones, including a new central pharynx. Each of the tail-pieces has one new pharynx; (ii.) has also one lateral pharynx-bud.

In another series (B) the planarians, cut into ten pieces, were kept ten days before being killed.

(*a*) The head-pieces died with but one exception. In this a pharynx cavity is formed with, possibly, a pharynx-bud.

(*b*) The second pieces except worm (ii.) show new pharynges regenerated.

(iii.) and (iv.) have developed a new central and four new lateral pharynges.

(i.) has a central and three lateral pharynges.

(*c*) The third pieces all have a new central pharynx and from one to three new lateral ones. In one piece the anterior pharynx is probably the old one.

(*d*) The fourth pieces show new pharynges regenerated at at each end of the pharynx cavity, there being old ones in the middle. (i.) and (iii.) have a new central pharynx and several new lateral posterior ones, and several old ones in the middle.

(ii.) differs in having other new pharynges besides the central one in the anterior part.

(*e*) The fifth pieces are similar to the fourth, having new pharynges at both ends, old ones in the middle.

(*f*) Two of the sixth pieces have four new lateral pharynges posteriorly, the central one and the three anterior lateral ones being old.

(iii.) has a central and two lateral pharynges, probably all of them new.

(*g*) All of the seventh pieces have a new central pharynx, old ones in the middle and new ones towards the posterior end.

(*h*) One worm of the eighth set shows a condition similar to those of the seventh set. The other has only new pharynges, a central and two lateral ones.

(*i*) The ninth pieces have two or three new pharynges, (i.) has a central one, while in (ii.) no central pharynx has regenerated. No old ones are left.

(*j*) Two of the tenth pieces have not formed new pharynges, the third has a new central and two new lateral pharynges.

In another series (C) the planarians were cut into the three regions of the body — head, pharyngeal and tail parts and then each of these were cut into very small cross-pieces; they were kept nineteen days and then killed. In the head pieces a new central and from three to five new lateral pharynges have developed.

The pieces from the pharyngeal region give no results as the pharynges are too advanced for one to be able to determine which of the pharynges are old and which are new.

One of the tail pieces had no pharynges developed. The others had a central and from four to six new lateral pharynges regenerated.

The only other study that has been made of the regeneration of *Phagocata gracilis* is that of Lillie in 1901.[1] He showed that "the power of regeneration of this genus is equal to that of *Planaria*." He also pointed out that in cross-pieces several pharynges regenerate simultaneously, although the more anterior ones are the most advanced. Each pharynx develops at first in a separate cavity of its own and the cavities fuse together to form a common pharyngeal cavity. We have also observed this mode of development. Lillie found that in lateral pieces new pharynges first arise on the old side of the piece and the new ones on the opposite, regenerating side do not appear until a branch of the intestine has extended into this region. The new pharynges connect at one end with this branch.

CONCLUSIONS.

The regeneration of cross-pieces from different levels shows the following relations.

1. Pieces anterior to the old pharyngeal chamber produce in the new tissue at the posterior end a new median pharynx and lateral pharynges that increase in number from before backwards.

2. Pieces containing only the old median pharynx develop new lateral ones from the sides of the pharyngeal chamber.

---

[1] Lillie, F. R., "Notes on Regeneration and Regulation in Planarians," *Amer. Jour. Physiology*, VI., 1901.

3. Pieces containing only some of the lateral pharynges develop a new median pharynx at the anterior end of the pharyngeal chamber and new lateral pharynges both in front of and behind the old lateral pharynges.

4. Pieces cut behind the old pharyngeal chamber develop a new median anterior pharynx and new lateral ones are added from before backwards.

5. Old lateral pharynges do not appear ever to be substituted for the old median pharynx if this is removed.

AN EXAMINATION OF THE PROBLEMS OF PHYSIOLOGICAL "POLARITY" AND OF ELECTRICAL POLARITY IN THE EARTHWORM.

T. H. MORGAN AND ABIGAIL C. DIMON.

The so-called "polarity" exhibited in the regeneration of animals has suggested the idea to a number of writers that the phenomenon might be related to, or the outcome of differences in potential in different regions; or, in other words, of electrical polarity. The term "polarity" itself, which has been generally adopted to express a sort of stereometric relation in the regeneration of living things, suggests in certain striking ways the polar relations observable in many electrical phenomena, and invites a direct comparison between the two.

The only experiments that have been undertaken to test directly this question are the recent ones by Mathews[1] on certain hydroids and on the tail of Fundulus. The interesting results reached by Mathews, while leaving the problem, so far as the main issue is concerned, still an open one, showed the importance of further examination of the subject. Mathews avoids, it is true, making a direct comparison between physiological "polarity" and the polarity present in electrical phenomena, and speaks rather of the rate of growth of certain regions in comparison with others; but if there is in reality any fundamental relation between the phenomena in question, we should expect to find some expression of it in the polar, or, more generally, the stereometric relations of the parts.

If, for instance, the development of a head at the anterior end of a piece and of a tail at the posterior end is connected with difference of potential in the two regions, we might hope to get evidence of this by means of the galvanometer. If this relation should be found to exist, there is a further opportunity of testing the validity of the conclusion in the case of axial heteromorphosis. For this reason we selected the earthworm for our study, since in the earthworm it had been shown by one of us that there regenerates from the anterior cut surface of posterior pieces not a head, but a tail. We should expect to find under these circumstances a reversal of the potential in this region, when compared with an anterior cut surface in the more anterior regions of the worm. The following pages give the results of our examination.

---

[1] Electrical Polarity in the Hydroids. A. P. Mathews. Am. Journ. Physiology, Vol. VIII, No. IV, Jan. 1, 1903, pp. 294-299.

METHODS.

Both Lumbricus terrestris and Allolobophora fœtida were used. Since the results appeared to be similar for both species, Lumbricus, being larger and showing on the whole greater differences of potential, was preferred when available. In all, sixty-four worms were used, the number of tests made upon any one varying from one to seventeen. Differences of potential were detected by a Rowland-d'Arsonval galvanometer, connected with a pair of non-polarizable electrodes. Since the regulation of these electrodes was found to be troublesome, and since they were liable to introduce a source of error into the readings, their manufacture and regulation had best be described. Two glass tubes about three inches long, somewhat smaller at one end, were plugged at the small end with kaolin or filter paper, moistened with normal (0.85%) salt solution. The plug extended about a half inch beyond the end of the glass tube. When made of kaolin, the end, after being used, could be easily broken off and re-formed from fresh material. When made of filter-paper, the tip, if it became contaminated from touching the worm, could be cut off. Above the plug each tube contained a saturated solution of zinc sulphate, into which projected through a cork a small amalgamated zinc electrode, connected by a wire with one of the poles of the galvanometer. Since the instrument was so sensitive that the slightest loss of equilibrium was at once registered by a deflection of the mirror, great care had to be exercised in keeping the junctions of wires and zinc electrodes dry, and in balancing the other elements of the electrodes. As the electrodes were found to deteriorate rapidly when used, it was necessary to examine them at frequent intervals, and when the deterioration became so great as to affect seriously the value of the readings on the worm, to readjust the parts. This could be accomplished in several ways, of which sometimes one and sometimes another was most effective. A fresh zinc electrode could be substituted, the zinc electrodes could be freshly amalgamated, or they could be polished by rubbing them with sandpaper. The current could sometimes be affected by moving one zinc so that a greater or less surface was immersed in the zinc sulphate. Putting fresh zinc sulphate solution into the tube nearly always produced a distinct effect. All these means of regulation proved more or less temporary, and often, when they all failed to balance the electrodes, fresh ones had to be made. The electrodes in which filter-paper was used proved much more constant and easy to regulate than the clay ones, and if they were often renewed, it seemed safe to employ them.

A positive deflection of the galvanometer meant that the potential at the right hand electrode was higher than that at the left hand, *i. e.*, the current through the galvanometer flowed from right to left. Since in nearly all cases the left hand electrode was placed on the worm anterior to the right hand one, a positive deflection meant that the current flowed through the galvanometer from the posterior to the anterior electrode.

The electrodes were applied, sometimes both to the dorsal surface of the worm, sometimes to the ends made by transverse sections through the body, and sometimes one to the surface, and the other to a cut end. The results will be considered according to the position of the electrodes.

ONE ELECTRODE ON THE SURFACE AND THE OTHER ON THE CROSS-SECTION.

It will be seen from the records given in the following selected tables that however general certain results appear to be, nevertheless some individuals show irregularities. This uncertainty in the results can be in part at least accounted for by the following considerations. Secretions in different regions of the body, the flow of blood from cut surfaces, the excretion of slime from the skin, local or general muscular contractions might all tend to affect the results. Such effects could sometimes be distinctly seen in an increase or decrease in the deflection of the galvanometer. It seemed possible that the presence or absence of food in the digestive tract might in itself or by stimulating the flow of digestive fluids influence the distribution of electric potential, but this was not observable from tests made with worms that had been starved for several days.

Experiment 1. Lumbricus terrestris starved two days.[1] Cut ends anterior at several levels. The left hand electrode was applied to the cross-section and the right hand electrode to the dorsal surface one-third to one-half inch behind the section. The zero point of the galvanometer was 27.1, and the effect of the electrodes varied, deflecting it between the limits 26.1 and 29.3, both of which deflections are less than those caused by the worm itself, and may therefore be disregarded. The galvanometer readings at different levels on the worm were as follows:

Cut at fourth segment....	a. 45.0+ (off scale)	Surface positive
Cut at fourteenth segment.	b. 35.5	" "
Successive sections between the fourteenth segment and the middle of the worm................	c. 45.0+ (off scale)	" "
	d. 42.5	" "
	e. 40.0	" "
	f. 43.5	" "
Successive sections posterior to the middle.........	g. 11.5	Cut end positive
	h. 34.0	Surface positive
	i. 32.2	" "

The readings are all definite and represent the state of affairs in a majority of the worms examined. From these data it will be seen that when a worm is cut in two it is found that in the anterior regions of the worm the anterior cut end of the posterior piece is negative with respect to the near-lying surface. In the posterior regions of the worm where there was more variation the differences in potential were usually less, and sometimes reversed in

---

[1] Since the results seemed not to be affected by the absence of food from the digestive tract, this specimen was chosen because a more complete series of sections were made from it than from any unstarved worm.

direction. Irregularities were often observed at about the fifteenth segment, where the male reproductive organs open to the exterior and the crop-gizzard region begins. Irregularities also occurred at the girdle. In the worm used for this experiment negative deflection of the galvanometer occurred only once, immediately behind the middle of the worm, but the positive deflection was diminished at the fourteenth segment and at the other cut ends in the posterior regions of the worm.

Experiment 2. Lumbricus terrestris. Cut end posterior. The zero point of the galvanometer was 27.3, and the electrodes caused a deflection to 27.5 at the beginning and to 30.0 at the end of the experiment, which does not affect the sense of the deflection for any reading. The galvanometer record for the different levels is as follows:

Successive sections from the posterior end to the middle of the worm...	a.	28.0	Cut end positive
	b.	19.5	Surface positive
	c.	23.0	" "
	d.	23.5	" "
Section about the middle..........	e.	24.0	" "
Section anterior to middle.........	f.	25.0	" "
Section at 23rd segment...........	g.	39.0	Cut end positive
Section at 15th segment...........	h.	20.0	Surface positive
Section anterior to 15th segment....	i.	17.7	" "

The surface has a higher potential than the cut end at all but two levels, one where only a few segments are cut off from the posterior end, where the difference of potential is small; and the other at the twenty-third segment. The reversal of current at the extreme posterior end occurred in all the worms in which a full series of sections was made. The twenty-third segment is between the fifteenth segment and the girdle. In this region two other worms showed possible cases of reversal of current, though the more usual condition was that the current was reversed at the fifteenth segment or at the girdle, or at both points, but between them flowed in the same direction as in the rest of the worm. The worm used in this experiment showed the same distribution of potential that is found in a majority of individuals, except at the fifteenth segment, where one-half of the worms tested showed a reversal of current, the other half agreeing with this experiment in showing no reversal.

From experiments 1 and 2 it may be assumed that when an earthworm is cut in two, the transverse section commonly represents a point of lower potential than the uninjured surface near to it. At the cut end chemical changes no doubt take place as a result of the fluids there set free, and of the general breaking down of tissues. These conditions might be expected to alter the electrical potential at the cut end, and presumably, where these changes are greatest, the alteration of potential will be greatest. Since, however, the transverse section is usually lower in potential than the uninjured surface near it, whether the section be at the anterior or posterior end of the piece, the difference in potential cannot bear any relation to the *kind* of regeneration that is to take place.

Having illustrated the more regular and more usual conditions of distribution of potential between surface and cross section, two illustrations of the exceptions to this condition, sometimes met with, will now be given. In the first a distribution of potential different from the average occurred throughout the whole worm. In the second, a short piece of a worm showed unusual conditions.

Experiment 3. Lumbricus terrestris, young. Cut end posterior. The zero point of the galvanometer was 27.4, and the electrodes deflected it to 26.3 at the beginning of this experiment, and to 28.9 at the beginning of the next experiment made that day. Some doubt may, therefore, be thrown on two of the readings given below—those at the girdle and at the fifteenth segment. The direction of deflection in these cases is probably correct, but the amount is small. The readings were as follows:

Posterior to middle..........	a	24.0	Surface positive
Just back of girdle..........	b	29.0	" "
Anterior to girdle...........	c	33.5	Cut end positive
	d	34.6	" "
At 15th segment.............	e	26.5	Surface positive
Anterior to 15th segment.....	f	32.8	Cut end positive
	g	36.0	" "
	h	34.9	" "
	i	35.0	" "

Here we see the cut end with a higher potential than the surface, except at the girdle and in front of the fifteenth segment, where the current is reversed. The worm was small and immature, but as other young worms gave the same sort of readings as the majority of mature worms, the peculiar results cannot be due to immaturity. In fact, it is difficult even to guess why this worm should give such different responses from the others. Another case was also recorded in which the cross section was anterior and of higher potential than the neighboring surface for a series of seven sections.

Experiment 4. Lumbricus terrestris. Piece one inch long from near the posterior end of the worm, with a cut surface at each end of the piece. Zero point of galvanometer, 28.6; electrodes, 30.0.

Electrodes at anterior end and middle of piece..........	26.3	Anterior cut end positive
Electrodes at posterior end and middle of piece..........	27.1	Surface positive
Electrodes at both ends......	27.0	Anterior cut end positive

In this experiment the anterior electrode is positive with respect to the posterior one, whether it be on an end or on a surface, and we get a constant direction of current from before backward. The worm from which the piece was cut gave the usual results for the other readings made on it. The piece was cut out by two consecutive cuts with no appreciable time between them, so that the freshness of the cut could not, as it does in other cases given later, influence the result. One other case resembled this one, while two cases showed conditions in which the middle was positive with respect to both ends, and three cases showed conditions in which the middle was negative with respect to both ends.

It has been stated that marked changes in the distribution of potenial between the surface and cross section often occur at the fifteenth segment and at the girdle region. When a worm is cut in two at the fifteenth segment the cut end has usually a higher potential than the surface either anterior or posterior to it, or when short pieces of a worm are cut with one end at the fifteenth segment, that end is positive to the other, whether it be an anterior or a posterior end of the piece. When, however, a worm is cut in two immediately anterior or immediately posterior to the girdle, the girdle has a lower potential than either cut end. Both these regions, therefore, show a state of affairs different from that in other parts of the worm. When worms were cut in two at a series of points it was found that at the fifteenth segment there was a change in direction of the current in twelve cases out of nineteen, and at the girdle in eight cases out of fourteen, four cases in which the change was not very pronounced being included in the first series, and two in the second. The change in direction of current, though by no means uniform, is rather more likely to occur than not, and may perhaps be connected with substances secreted by the organs at these levels.

If the distribution of potential in the earthworm resembles the distribution of potential in a resting muscle, we might expect that the difference of potential between the electrodes would vary according to the position of the electrodes. A series of experiments was tried, in which one electrode was kept stationary at a transverse section, and the other moved along to different positions—usually one near, one half way between the ends, and one on the skin at the end opposite the transverse section. Great variation in the deflection of the galvanometer was always observed for these different positions, but it was by no means regular. The most common case was that the deflection was greatest when the electrodes were near one another, decreasing as they moved away, and sometimes even changing to an opposite direction when they were at opposite ends of the worm. In this series of experiments we have not only the complicating conditions already mentioned, but also the factor of resistance which would be approximately proportional to the distance between the electrodes, and if appreciable would modify the results in the way stated. The problem is one of greater complexity than that of the distribution of potential in the comparatively homogeneous tissue of the muscle, where the resistance is small. If the earthworm were homogeneous as regards electrical conductivity, and a difference of potential were set up by means of a transverse section, the point of lowest potential would be in the middle of the cut end, and of highest potential at the opposite end of the worm. If, however, resistance varied in different parts of an unhomogeneous tissue, the difference of potential observed between two points would be a resultant between a tendency to a regular rise of potential and an irregular distribution of resistance, and the recorded distribution of potential would, therefore, be irregular. In point of fact, however, as mentioned above, other causes of irregularity may be added to those due to resistance, and many arrangements of potential were observed in the seventeen cases tested.

ELECTRODES APPLIED TO TWO POINTS ON THE SURFACE.

Experiment 5. Lumbricus terrestris. The zero point of the galvanometer was 28.6, and the electrodes deflected it to 27.9 or 27.8, in an opposite direction from deflections caused by the worm. This experiment was made in order to see what the electrical conditions are on the surface of the worm. Three readings were taken, as follows:

a. Earthworm cut in two at the middle. Posterior piece. One electrode on the surface at the section and the other on the surface, posterior to the section, but near it	30.3	Posterior positive
b. Same worm. Short piece cut from posterior half of animal. One electrode on the surface at the anterior section, and one on the surface posterior but near	33.9	" "
One electrode on the surface at the anterior section, and one on the surface at the posterior section.	35.0	" "

The direction of current was the same as would be expected if one electrode were applied directly to the cut end, and the other to the uninjured surface near the end. On the assumption that at any level the conditions of the surface fairly represent those in the interior of the worm, by testing the distribution of potential at the surface of an uninjured specimen it may be possible to get some idea of the distribution within the worm. This was done in the following experiment.

Experiment 6. Lumbricus terrestris. Both electrodes applied to the surface. The zero point of the galvanometer was 26.9, and the electrodes varied from 26.7 to 22.0 after the third reading, when they were regulated to 24.3, and at the end registered only 26.0. The readings of the galvanometer were as follows:

One electrode at anterior end, the other near	32.0	Head end negative
One electrode at anterior end, the other at middle	30.0, then up, off scale.	Head end negative
One electrode at anterior end, the other at posterior end	16.0	" " positive
One electrode at posterior end, the other at middle	28.0	Tail end positive
One electrode at posterior end, the other near	18.0 22.5	" " negative

It is difficult to explain these data so that they are consistent. There is no one point that has a high potential relative to all others, though the deflections are sufficiently strong to indicate that they are not due to variability in the electrodes themselves. The variation may perhaps be due partly to local muscular contraction and partly to the excretion of slime at points on the surface, for in *Allobophora,* where the body cavity fluid extruded through the dorsal pores is yellow and noticeable, its excretion was observed to have a great effect upon the deflection of the galvanometer.

Different worms, too, show the greatest differences as to their reactions when electrodes are touched to different parts of their surface. In general, the two ends tend to have a lower potential than other parts of the surface, and the middle tends to have a higher potential with respect to points on either side of it. At the girdle and at the fifteenth segment, however, the results are more definite, as is shown in the two following experiments.

Experiment 7. Lumbricus terrestris. Zero point of galvanometer, 28.6; deflection caused by electrodes to 30.5.

One electrode at girdle, the other anterior to it, and near	20.5	Girdle negative
One electrode at girdle, the other posterior to it, and near	39.0	" "

Experiment 8. Lumbricus terrestris. Zero point of galvanometer, 28.6; deflection caused by electrodes to 30.0, at end of experiment.

One electrode at 15th segment, the other anterior to it and near	29.8	15th segment negative, probably
One electrode at 15th segment, the other posterior to it and near	31.5	" " "

The girdle is definitely of a lower potential than the surface near it, anterior or posterior, and this was found to be the case for four worms tested. At the fifteenth segment the difference was not so great, and though this region was negative with respect to a surface posterior to it, with respect to one anterior it was only very slightly, or, perhaps, not at all so. In another worm the fifteenth segment was evidently positive with respect to a surface anterior to it.

ELECTRODES APPLIED AT TWO TRANSVERSE SECTIONS.

Experiment 9. Lumbricus terrestris. Pieces cut out from worm. The zero point of the galvanometer was 27.3. The electrodes deflected it to varying amounts. In two cases, namely the fourth and the seventh readings in the table, where the electrodes deflected the galvanometer to 26.1 and to 29.5, respectively, these deflections come near the readings given by the worm. In the other cases it is not necessary to take deflection caused by the electrodes into account, since they would not affect the direction of the reading. The data are as follows:

(a) Anterior half of worm, both ends cut (long piece)	16.6	Anterior positive
(b) Short piece from anterior part of anterior half of worm, the posterior end cut somewhat later than the anterior end	22.3	" "
(c) Same. Anterior end freshly cut	32.5	Posterior positive
(d) Short piece from middle part of anterior half of worm, posterior end more freshly cut	27.1 27.0 27.9	" "
(e) Same piece, anterior end freshly cut.	36.2	" "
(f) Short piece from anterior part of posterior half of worm	33.0	" "
(g) Short piece from middle of posterior half of worm	28.5 24.0	Anterior end positive probably
(h) Anterior half of (g), posterior end freshly cut	22.0	Anterior positive
(i) Posterior half of (g), anterior end freshly cut	34.0	Posterior positive

In this worm, when the two ends were cut at approximately the same time, which happened in (a), (f) and (g), the piece from the anterior half had its anterior end positive, and the two pieces from the posterior half had their posterior ends positive. In the majority of worms tested, when the two ends of a piece were cut at the same time, the anterior end was positive, regardless of the position of the piece on the worm.[1] If, however, the two ends were cut at different times, which in this worm occurred in six pieces, the end which had been cut most recently generally had a lower potential than the other. Since the testing with the electrodes on a transverse section and an uninjured surface near that section the end was usually found to be at a lower potential than the surface, the fall of potential was supposed to be due to changes accompanied by the escape of body fluids at the cut end. Since, when the electrodes are on two transverse sections, the one that is more recently cut is of a lower potential than the other, it would appear that the causes that determine the fall of potential are such as decrease in the course of a short time. The fluids that escape from the cut end dry rather rapidly, whereas the tissue cells, breaking down, are not built up for several days. The first fall of potential, then, is probably largely due to escape of blood or other fluids from the section, while the slighter permanent effect may be due to the breaking down of tissue cells. When there was but a short time between the two cuts the lower potential did not always occur at the fresher one, which may have been partly because fresh fluids were still coming from the earlier section. In the anterior half of the worm, also, there was great irregularity, which may be partly due to different digestive fluids at different regions of the digestive tract producing varying electrical activities.

---

[1] When, however, the pieces were long (one-half the worm or more), in a majority of cases the posterior end was positive with respect to the anterior

The differences of potential between two transverse sections are probably a resultant of the factors that cause difference of potential between a section and a surface. If a piece be cut out from an earthworm by two transverse sections, there will be a difference of potential between each end and the surface between the ends. If the differences of potential between each end and the surface are equal and opposite they will balance one another, and there will be no difference of potential when electrodes are applied to the two ends. If, however, the differences of potential between the two ends and the surface are unequal, their resultant will determine a difference of potential between the two ends. This is illustrated by experiment 10.

Experiment 10. Lumbricus terrestris. Short piece. The zero point of the galvanometer was 28.6, and the electrodes registered slightly above this at the beginning of the experiment. The readings were as follows:

(a) One electrode applied to anterior end, the other to the middle of the piece.......................... 25.0
(b) One electrode applied to posterior end, the other to the middle of the piece.......................... 30.8

(c) One electrode applied to one end, the other to the other end of the piece.............................. 28.0

If we disregard the deflections caused by the electrodes, the following are the departures from the normal: (a.) = – 3.6, (b.) = +2.2, (c) = – 0.6. Theoretically, if (c.) were the resultant of (a.) and (b.), it would equal – 1.4, but considering how variable the conditions were always found to be, – 0.6 presents a fairly close agreement. In all other cases but one, where similar tests were made, the results agreed in like manner with the theory.

REGENERATING WORMS.

In addition to the readings made from worms that had been freshly cut in two, a series of readings were made on worms in which the process of regeneration had proceeded for a number of days. The regenerating worms were divided into four groups: (1) those in which a few anterior segments had been cut off and regeneration was taking place at the anterior end of the long piece; (2) those in which the worm had been cut in two in the middle and regeneration was taking place at the posterior end of the anterior piece; (3) those in which the worm had been cut in two in the middle and regeneration was taking place at the anterior end of the posterior piece; (4) those in which the worm had been cut in two at the fifteenth segment and regeneration was taking place at the anterior end of the posterior piece. They were allowed to regenerate from twenty-five to thirty-two days, and in the course of that time five or six tests were made on most of them at intervals of a few days. One electrode was applied to the regenerating tip, and the other to the old surface a short distance from the tip. In all one hundred and fourteen readings were recorded, the results of which may be summarized as follows:

Group (1)........	31	cases, end positive;	9	cases, end negative
Group (2)........	20	" " "	18	" " "
Group (3)........	15	" " "	11	" " "
Group (4)........	8	" " "	2	" " "
	—		—	
Total..........	74	" " "	40	" " "

If we regard only the readings made when regeneration had proceeded more than twenty-one days, in thirty-six cases the end was positive with respect to the surface, and in eleven cases it was negative, a much more definite result than when all cases are considered.

The conditions in a regenerating tip do not, therefore, agree with those at a freshly cut end, for the current flows in an opposite direction. To be sure, the processes occurring during regeneration are not the same as those occurring during the breaking down of tissues, and the latter may be the predominant ones immediately after a cut is made. The subject, however, needs further investigation before the causes for this reversal can be more than surmised.

ELECTRICAL POLARITY AND RATE OF REGENERATION.

If we look for a relation between electrical polarity in the worm and rate of regeneration, as Mathews has suggested, we find it as difficult to demonstrate as the difference between electrical and physiological polarity. If the average deflection of the galvanometer was greater at certain levels where regeneration is known to be rapid than at other levels where it is slow, the connection would be established. For instance, the regeneration of a tail at the posterior end of a worm when only a few posterior segments are cut off, is exceedingly rapid, whereas the regeneration of a heteromorphic tail at the middle of a worm is very slow. The average deflection of the galvanometer in the former case for three readings is, however, 3.4, with the cut end positive instead of negative in every case. In the latter case the average deflection for seven readings is 3.6 (with the end negative), with no extreme readings to bring it up. The region in the middle of the worm, where a tail is to regenerate from the posterior end of the anterior piece, gives an average deflection of 4.7 for seven readings from different worms. When five or six segments are cut from the anterior end of the worm the average deflection for seven cases was 4.6 at the anterior end of the long piece. At the posterior end of the short piece, regeneration would be very slow, and at this end only two readings were made, one giving a very slight result, the other deflecting the galvanometer to 5.2.

If we attack this subject by another method, namely, by making a direct comparison between two freshly-cut ends on one worm, the results are equally indefinite. When five or six segments were cut from each end of the worms, of a series of fifteen readings on different worms made with one electrode at the anterior-cut end and the other at the posterior-cut end, the posterior end was positive in eleven and the anterior end positive in four. When the worm was cut through the middle and at the posterior end, the posterior end was positive in three cases, and the middle in one case. When the cuts were made through the middle and anterior end, in five cases the middle was positive, and in two the anterior end positive. From these considerations it would therefore appear that no invariable connection between rate of regeneration and electrical polarity exists in the earthworm, at least as measured on a freshly cut surface.

From the foregoing experiments we conclude:

(1) That a freshly cut end of an earthworm is generally negative with respect to a near-lying uninjured surface.

(2) That the freshness of the cut surface has an important influence in determining the amount of difference of potential.

(3) That the result is often complicated by the presence of secretions or exudations on the surface, or by the presence of certain organs at the cut end, or by the contractions of the worm, etc.

(4) That in the region of the girdle and also in the region of the fifteenth segment (near which the crop and gizzard lie), the results are often different from those elsewhere.

(5) That there is no apparent relation between the differences in potential at freshly cut surfaces and the kind of regeneration (head or tail) that occurs.

(6) That cut surfaces from which heteromorphic growth would take place show the same sort of differences in potential as those from which orthomorphic regeneration occurs.

(7) That the differences in potential present when a cut surface is exposed can probably be accounted for by the chemical changes taking place at the surface; and these need have, and do

not appear to have, any relation to the kind of regeneration that takes place.

(8) That when, on the other hand, the cut surface is allowed to heal, and when later a new structure has begun to appear, the differences in potential between the new and the old parts (*as measured on the surface only*) are not such as can be made to account for the difference in the kind of part (head or tail) that is regenerating. Here also many complications enter into the result and make it difficult to draw satisfactory conclusions.

(9) No definite relation was found between the rate of growth and the fall of potential between an uninjured surface and a cut end.

THE REGENERATION OF A HETEROMORPHIC TAIL IN ALLOLOBOPHORA FOETIDA.

ABIGAIL CAMP DIMON.

In a paper by Professor Morgan[1] an account was given of anterior regeneration from three different levels in earthworms. The results seemed to show that the internal factor determining the formation of a heteromorphic tail might be the presence of the stomach-intestine at the regenerating surface, and at Professor Morgan's suggestion and under his direction, the following experiments were undertaken. An attempt was made to test this view by means of more exactly localized sections made near the level of the beginning of the stomach-intestine.

In *Allolobophora foetida,* the species used, the œsophagus extends to the fifteenth segment, the crop lies in the fifteenth and sixteenth, the gizzard in the seventeenth and eighteenth, and at the nineteenth begins the stomach-intestine, which extends posteriorally through the rest of the worm. The external openings of the vasa deferentia on the fifteenth segment served as convenient landmarks for determining the level of the section. The worm was cut in two, the short anterior piece dropped into alcohol, and its number of segments counted so that the exact level of the cut could be recorded. The posterior piece was then left to regenerate from forty-eight to one hundred and twenty days, when it was killed, and sections made for study. In some cases a new head, and other cases a new tail regenerated from the anterior end of the posterior piece. In the regenerating head the new stomodæum usually did not open into the old digestive tract, which closed anteriorly, and no definite pharynx formed. A dorsal brain, connected with the ventral nerve cord was usually present. Since these conditions represented the most usual form of head regenera-

---

[1] Experimental Studies of the Internal Factors of Regeneration in the Earthworm. Arch. für Entwickelungsmech. der Organ. Bd. XIV. pp. 562–591.

tion at the levels of these experiments, the cases in which they exist are classified in the table as a separate group under Head A. Cases where the brain lies anterior and even ventral to the level of the digestive tract; where the nerve cord ends without forming a brain; or where there is no mouth invagination, though the brain is well developed, are classified as Head B. The heads of group B look less like a normal head than those of group A, and yet are very evidently to be classified as heads rather than as tails. The distinctive features indicating a heteromorphic tail are the formation of a number of segments, the opening of the digestive tract to the exterior through a new anus, and the ending of the nerve cord ventrally, without a brain. Tails possessing these features are put in group A, while those in which any of these features are absent, are put in group B.[1]

In all, one hundred and seventeen worms were examined, with the results given in the table. The number of the segment given at the head of each column locates the level at which the worm was cut in two, and both the actual number of worms and the percentages are given under each class.

	12th–13th Segment		Between 14th Segment and 15th Segment		Between 15th Segment and 16th Segment		Between 16th Segment and 17th Segment		Between 17th Segment and 18th Segment		Between 18th Segment and 19th Segment		Back of 1 th Segment	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Head, A..	2	100	7	70	11	46	7	59	6	46	6	18	...	.....
Head, B..	...	....	3	30	11	46	4	33	6	46	20	61	4	17
Tail, B...	...	....	....	....	....	....	....	....	....	....	1	3	5	22
Tail, A...	...	....	....	....	....	....	....	....	....	....	1	3	7	30.5
Uncertain	...	....	....	....	2	8	1	8	1	8	5	15	7	30.5
Total ..	2	100	10	100	24	100	12	100	13	100	33	100	23	100

---

[1] In only twenty-four cases out of one hundred and seventeen did the old digestive tract open to the exterior through the new mouth or anus. This occurred ten times in head regeneration, ten times in heteromorphic tail regeneration, and four times in cases classified as uncertain. Seven of the twenty-four cases occurred when the worm was cut in two in front of the sixteenth segment, and the other seventeen when it was cut behind the eighteenth segment.

Since there were but few worms cut further back than the eighteenth segment, and since the stomach-intestine begins at this level, all the observations made on worms cut posteriorly to the eighteenth segment were brought into one class. It is worth noting, however, that of the four heads regenerating at these levels, three formed from worms cut at the nineteenth segment, while the fourth formed from a worm in which the exact level of the cut was not noted. The percentages in the different classes, though based on a small number of cases, yet bring out clearly one or two points. When a worm was cut in two in front of the stomach-intestine, in no case was a heteromorphic tail formed. The percentage of cases in which a head was formed grows less as the section is made further back on the worm, the fall of percentage being very great immediately behind the gizzard. This tends to support the hypothesis that the formation of a heteromorphic tail is favored by the presence of the stomach-intestine near the cut end, though when the section is not more than one segment back of the gizzard a head is sometimes formed.

Though the preceding experiments seem to show that the development of a heteromorphic tail is connected with internal structures in the worm, they leave untouched the question of the kind of regeneration that takes place from posterior ends of anterior pieces cut anterior to the stomach-intestine. This point should be determined, and I hope in the future to undertake a set of experiments in which the posterior regeneration from anterior pieces will be observed.

A CONTRIBUTION TO THE EMBRYOLOGY OF HYLODES MARTINICENSIS.

BY

LILIAN V. SAMPSON.

WITH 2 PLATES AND 17 FIGURES IN THE TEXT.

TABLE OF CONTENTS.

PAGE
INTRODUCTION 473
I. EXTERNAL CHARACTERS 474
  1. Segmentation 474
  2. Embryonic stages 475
II. ORGANOGENY 477
  1. Origin of Organs 478
  2. Digestive Tract 478
  3. Heart and Blood-Vessels 489
  4. Excretory and Reproductive Organs 494
  5. Tail 495
  6. Dermal Fold. Beak. Sense Organs 496
  7. Comparison with Other Forms 497
SUMMARY 501

For many years the general outline of the embryonic history of Hylodes Martinicensis has been known. Since the early seventies, it has been described as the West Indian tree-frog, with no aquatic tadpole stages, but with an embryo developing within the egg membranes as far as the adult form,—the embryo being provided with a large supply of yolk food, being devoid of gills, but with a highly vascular and, therefore, it was believed, respiratory tail. I am fortunate in having at my disposal a good series of the eggs of Hylodes, from which I have studied the development in more detail.

I am glad to take this opportunity of acknowledging my indebtedness to Professor T. H. Morgan for the material which he has collected and obtained from others at different times, and for his help and suggestions in the work.

The eggs that I shall describe were collected by Professor Morgan and Dr. Conant during two or three seasons, in the month of July, on expeditions of the Marine Laboratory of the Johns Hopkins University to Jamaica. The eggs were found at Bog Walk, on Blue Mountain Peak, and at Port Antonio. On Blue Mountain Peak they occur at a high elevation (about 6000 feet) in very damp localities, where the rainfall is very heavy.

Andrews, 92, reports that "these tree-frogs are found all over the island; at Port Antonio the breeding season seemed to be past July 20; while at Manchioneal, specimens with very large ovarian eggs were taken July 24."

I wish also to thank Mr. E. L. Griffin for a set of eggs collected at Cinchona (an elevation of 5100 feet).

The eggs are found imbedded in a clear gelatinous substance, in masses of about an inch in diameter, under loose stones or logs. During their development, the adult frogs are to be found near them. It appears, from the preserved material, that, in young stages at least, all the eggs of a mass are uniformly advanced in development.

I. EXTERNAL CHARACTERS.

1. SEGMENTATION.

The segmentation stages that were obtained are sufficient to show the general character of the division of the egg. In spite of the large quantity of yolk, the segmentation is holoblastic. The first furrow usually divides the egg in half, and the second comes in at right angles to the first (Plate I, Fig. 1[1]), but in one egg the first furrow has cut off not more than one-third of the egg from the rest. I have a number of eggs in the two-celled stage in which the second furrow is deeply marked on the upper pole, but extends only about half way around the egg (Figs. 1 and 2). I have no eggs that show how the third furrow comes in, or whether it appears before the second furrow is completed. In later stages the upper hemisphere divides more rapidly than the lower, as shown in Figs. 3 and 4. It has recently been found in the large egg of Desmognathus fusca, with a segmentation similar though even more unequal than that of Hylodes, that there is no equatorial furrow; nor is the third furrow equatorial in Triton cristatus, or constant in Salamandra maculosa. Later stages of Hylodes (cf. Figs. 3 and 4) show the smaller cells at the animal pole, the larger, yellower, more yoke-laden cells at the other pole. The next link in the history (Fig. 5) is the stage where the bastopore is closing in over the yolk-plug.

---

[1] The figures on the two plates are indicated by consecutive numbers, while text figures are marked by a letter of the alphabet.

2. EMBRYONIC STAGES.

Among the embryonic stages, I have material for a more complete history, especially in the later development, than the egg-stages have shown. For convenience in naming the stages, I have numbered them in order, although, owing to some breaks in the series, consecutive numbers do not represent equal advances in development. Since the ages of the preserved eggs are not known, I have compared the eggs with figures of embryos that were watched during development, and whose age was noted. The comparison shows that stages VI, XIII, XIV, and XV, correspond nearly with the embryos under observation which were labelled "7-8 days, about 12 days, just hatched, one hour old." As I have indicated, the former accounts of Hylodes have shown that the tree-frog at no time exists as a free-swimming larva, but that it develops enclosed within a membrane, which is not ruptured until the frog has reached a stage essentially like the adult, though sexually undifferentiated. When the gelatinous secretion about the preserved eggs has been removed, the vitelline membrane is found to be a tough transparent envelope through which the embryo can readily be seen. The eggs are of great size owing to a large amount of yolk, and are at first unpigmented. As the small, colorless embryo develops, it encircles the yolk more and more, while, at the same time, the yolk diminishes in quantity.

In stage IV (Figs. 8, 9), the diameter of the egg preserved in alcohol measures a little less than 3 mm., and the embryo is about one-half the length of the circumference. Both pairs of legs are present as knobs at the sides of the embryo; the tail also is scarcely more than a knob, although slightly lengthened and turned to the right or to the left side. In this stage the tail usually covers the anal opening, though the posterior rim of the anus still shows on the side from which the tail is turned away. The primary divisions of the brain are seen, the auditory vesicles are very distinct, and the optic cups are discernible. The embryo lies flat upon the egg so that its contour is scarcely raised above the level of the yolk. In stage III, the tail and legs are shorter than in stage IV, and on either side of the neck of the embryo are four deep parallel grooves, the ectodermic depressions which lie opposite the visceral pouches. In stage II (Fig. 7), the anus is seen at the posterior extremity of the embryo, and the tail is not formed, but the rudiments of both pairs of legs appear at about this stage. The optic vesicles show as diverticula from the brain. In stage I (Fig. 6), the blastopore is just closing, the divisions of the brain are not seen, but the head end of the embryo is broader than the trunk.

As development advances beyond stage IV, the legs of the embryo elongate, the area of the tail increases, and the brain and sense organs are further differentiated. At about stage VI (Fig. 10), pigment appears at isolated points over the dorsal surface of the embryo and in a dermal fold which covers the base of the anterior legs.

In stage IX (Plate II, Fig. 11), the eyes are large and heavily pigmented. The olfactory pits are plainly seen in an anterior view of the head, and are so far apart as to show from the side. The anterior legs lie close to the head (reaching well to the middle of the eyes when looked at from above). They are covered to the foot by the pigmented dermal fold. In some views, the outlines of toes can be seen. The tail is nearly twice as long as the hind leg, and is spread out under the egg membrane in a thin vascular sheet. The axis of the tail divides it into more or less unequal parts, the part lying towards the head end of the embryo being smaller than that lying on the opposite side of the axis. The tail is not pigmented, but the pigment in the embryo has increased in density, and is extending over the yolk.

Between stages IX and XII, the egg as a whole increases in size. and the embryo manifestly develops at the expense of the yolk. It is possible to see the increasing complexity of the brain until a period when the pigment renders the skin opaque. The fore-legs, as they increase in length, grow around the head (meeting ultimately under the chin), and the dermal fold, by which they have been partially covered, recedes. The hind-legs grow around the yolk, until they almost touch the fore-legs, and the fingers and toes become jointed and provided with suckers. The area of the tail increases. By the time stage XII (Plate II, Fig. 12) is reached, the embryo with the exception of the tail is uniformily though not deeply pigmented; in the tail, there are only a few scattered pigment cells. The lower lip, or rather the chin, is very thick, so that the head is less flat than before; on the upper lip is a single median black horny protrusion or beak.

In stage XIII (Plate II, Fig. 13), the frog no longer appears to be encircling an egg, but what yolk remains is incorporated in the body. Near each thigh is a pale triangular area, raised slightly above the surrounding surface. This is seen again in XIV (Fig. 14), where similar raised patches show on the sides of the neck.

The embryo in stage XIV has hatched, and looks like a somewhat bloated miniature adult frog. It measures 2.3 cm. in length; the adult frog measures 5 to 6 mm. The boundaries of the lymph spaces appear as pale lines in the skin, forming two seams on each side of the embryo and a triangle around each fore-leg, joined by two seams near together across the ventral surface. The chin is still very thick, and the hard tip of the upper lip persists. The tail is leaf-like and of considerable size, though much smaller in proportion to the body than in previous stages. The earlier observers record that the tail disappears during the first day, or even within a few hours after hatching. The pigment of the embryo in stage XIV is much denser than in preceding stages, but is not arranged in the pattern characteristic of the adult.

Stage XV differs externally from XIV in that the tail is reduced to a small thick knob.

If Hylodes be compared in external appearance with most of the forms of the group to which it belongs, with Rana, for example, it is seen to differ from the outset and throughout the immature stages much more than in the adult stage. The egg differs not only because of its great size, but in the absence of pigment. When the embryo of Hylodes first appears, it is curved ventrally, like the embryo of a Ganoid, over the bulky yolk, and obviously never has a dorsal flexure. When it begins to show its true affinities, it resembles, in the shape of the head, in the prominence of the eyes and in the presence of all four extremities, the frog stage of Rana, rather than the larval tadpole. Only the holoblastic cleavage and presence of the tail betray its relation to the larval form. In short, the usual Anuran metamorphosis is replaced in Hylodes by a direct development. The same fact is borne out by the internal characters.

II. ORGANOGENY.

The organogeny has been studied to some extent from dissections, but for the most part from sagittal and cross sections. In so-called "cross sections," the embryo, on account of its curvature about the yolk, is not everywhere cut transversely; the preparations have purposely been cut in various planes through the lateral axis of the egg, in order to get transverse and horizontal sections of different parts. (The left side of the figure is in each case the right side of the embryo.)

Owing to the large quantity of yolk in the younger embryos, it has been difficult to prepare good series of sections. Eggs killed in Perenyi's fluid are more readily saturated with paraffin and cut better than those killed in picro-sulphuric acid or picric alcohol, but the most satisfactory preparations have been obtained from specimens preserved in a fixative containing picric acid and (after dehydration) left in cedar oil for about 12 hours, in cedar oil and paraffin about 5 hours, and in paraffin (at a melting point of 49° C.) about 8 hours. The sections can be cut of any thickness from 5μ upwards. They were stained on the slide, because it is difficult to orient the embryo in the microtome after surface staining.

Eggs that are well preserved in formalin, make fairly good sections and can be readily cut after two or three hours in paraffin.

1. Origin of Organs.

My material is insufficient to more than suggest a few points in regard to the origin of the organs.

Sections of an egg of the stage of Fig. 5, showing the thick lips of the blastopore and the large yolk plug, and sections of a later stage, where the yolk plug is reduced and receding, are similar to sections of the corresponding stages of Rana. In sections of stage I (Fig. 6), the neural plate is solid, recalling the condition in Teleosts and bony Ganoids.

The notochord appears in a few sections in the posterior region of an embryo of stage II as an evagination of the dorsal wall of the archenteron (Fig. A, n) and the same condition is indicated in younger eggs. Anterior to the section shown in Fig. A the entoderm is continuous over the archenteric cavity, ventral to the notochord, but posterior to this section the entoderm does not appear to be continuous ventral to the notochord.[1]

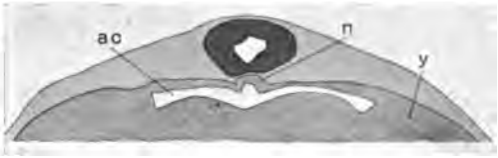


Fig. A. Cross section of stage II, through the posterior part of the embryo, to show the origin of the notochord. ac, archenteric cavity; n, notochord; y, yolk-mass.

2. Digestive Tract.

a. Alimentary Canal.—In stages midway between the first and last of the series, the chief organs derived from the archenteron are definitely established, and the alimentary tract is clearly divided in relation to the yolk-mass, into three regions, anterior, posterior and middle (see Fig. F).

At an early stage, the broad low archenteric cavity is continuous from one end of the embryo to the other (stage II, Fig. B1, ac). The floor of the cavity is the enormous yolk-mass, which is met abruptly at the sides by the thin roof of entodermic cells filled with yolk.

In the same stage (II, Fig. B1), the mesoblast is beginning to separate into splanchnic and somatic layers; as the process continues and the cœlom (Fig. B1, 2 bc) increases, the lumen of the archenteron becomes narrower (Fig. B2, ac), and in the middle region it is completely obliterated when the splanchnic layers of the two sides have met and form the mesentery which suspends the now solid yolk-mass (Fig. B3, m). After stage V, the yolk-mass in this condition occupies the region of the embryo between the more differentiated anterior and posterior portions of the gut (Fig. F, stage VI, cf. Fig. C, stage VIII).

---

[1] Cf. Bufo lentiginosus, where the conditions are similar, except that one layer only is contributed by the chorda-entoderm to the notochord in the middle region of the embryo.—King, 03.

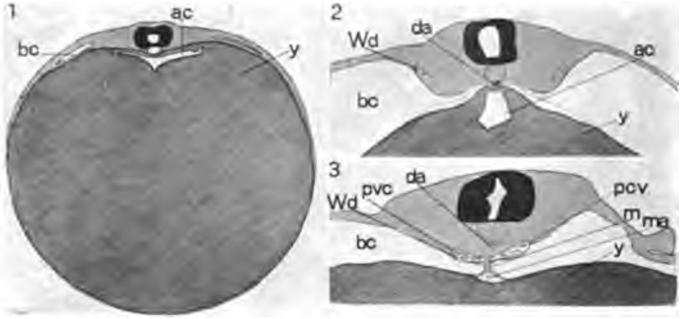


Fig. B. Cross sections of three stages, to show the fate of the archenteric cavity. 1, stage II (drawn to smaller scale than 2 and 3); 2, stage IV; 3, stage VI. ac, archenteric cavity bc, body cavity; da, dorsal aorta; m, mesentery; ma, mesenterial artery; pcv, posterior cardinal vein; pvc, posterior vena cava; Wd, Wolffian duct; y, yolk-mass.

There are no intermediate stages between I and II to show the fate of the blastopore and the history of the anus. In the posterior region of an egg younger than stage I, before the notochord is formed, and in stages I and III, posterior to the notochord, there appears a dorsal evagination of the archenteron, which suggests the neurenteric canal, though not actually traced into the neural tube in the stages in which it is hollow. In stage II the anus is formed, and in IV the differentiation of the hind-gut is in progress. The lateral walls of the posterior part of the broad low archenteric cavity covered by the splanchnic mesoderm fold under until they meet over the yolk-mass (Fig. D1, 2, ac, g). The process of infolding appears to take place from behind forwards, forming a tube that opens posteriorly by the anus, and anteriorly is continuous with the archenteric cavity of the middle region. Thus the cloaca, the portion of the gut from the anus to the opening of the Wolffian ducts, is established, and through all stages retains its lumen.

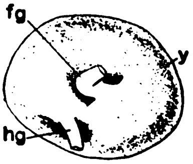


Fig. C. Yolk-mass of an embryo of stage VIII, after the embryo has been taken away, seen from the dorsal side, to show the relation between the yolk-mass and the anterior gut and posterior gut. fg, foregut; hg, hind-gut; y, yolk-mass.

As the cloaca is formed, the lumen of the archenteron is being obliterated in the middle region, and in later stages, the cloaca leads to a rod of yolk-cells, usually solid[1] at every point except near the middle yolk-mass. The rod, little by little, from behind forwards, is separated from the central mass of yolk, and covered ventrally by the splanchnic mesoderm (Fig. E, F, g). A continuation of the mesentery which slings the yolk-mass in the middle region supports the anterior portions of the rod or gut (Figs. B3, E1, m); but no mesentery is present dorsal to the cloaca. The posterior gut at stage VII has been separated from the yolk and completely surrounded by the mesentery as far forward as the level of the anterior limit of the hind legs. The gut up to this time lies in the median line. After stage VII, additions to the gut are still made from the dorsal part of the yolk-mass, but on the left of the median line (cf. Fig. C, hg). As the process goes on, the gut necessarily encroaches upon the middle region.

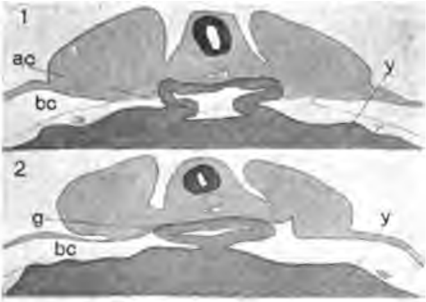


Fig. D. Cross sections of stage IV, to show the formation of the posterior gut: 1 anterior to 2 (and posterior to A2). ac, archenteric cavity; bc, body cavity; g, gut; y, yolk-mass.

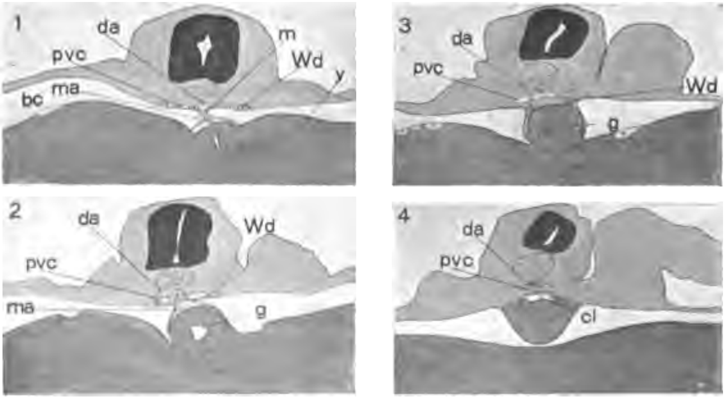


Fig. E. Cross sections of stage VI, to show the connection between the posterior gut and the yolk. 1 to 4 from before backwards (posterior to Fig. B3). bc, body cavity; cl, cloaca; da, dorsal aorta; g, gut; m, mesentery; ma, mesenterial artery; pvc, posterior vena cava; Wd, Wolffian duct; y, yolk-mass.

The first steps in the development of the anterior end of the digestive tract are not apparent in my series. In stage II the anterior portion of the archenteric cavity is very large. In III the mouth cavity and the pharynx are already formed; posterior to the pharynx the walls of the archenteron, covered by splanchnopleure, are folding under from the sides. Thus the œsophagus is formed, and in stage III is ventrally entirely separated from the yolk-mass (cf. stage V, Fig. G2), but leads posteriorly into the archenteric cavity of the middle region. After stage V, the œsophagus being full of yolk, is for a time nearly solid (stages VI and VII), after the mesentery is first completed. The distinction between stomach and intestine is not apparent, except that the lumen is greater in the definitive stomach; the intestinal part, near its connection with the yolk-mass in thrown into a loop (Fig. H8, g).

---

[1] I have seen sections of the tadpole of Rana, in which the archenteron at an early stage is closed for a short distance.

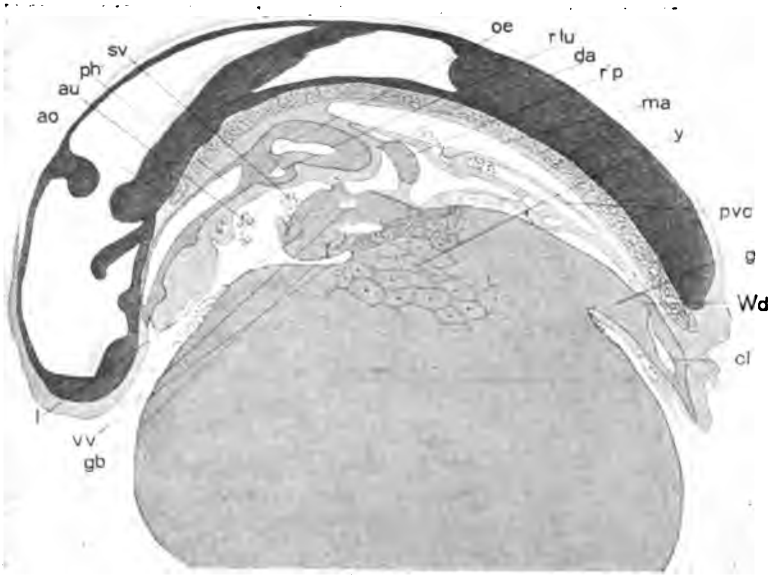


FIG. F. Sagittal section of stage VI. ao, aorta; au, auricle; cl, cloaca; da, dorsal aorta g, gut; gb, gall bladder; l, liver; ma, mesenterial artery; oe, oesophagus; ph, pharynx pvc, posterior vena cava; rlu, rudiment of lung; r'p, 1st rudiment of pancreas; sv, sinus venosus; vv, vitelline vein; Wd, Wolffian duct; y, yolk-mass.

Liver.—In stage III, when the lateral walls of the archenteron are folding under to form the gut, the archenteron is extended into a diverticulum, from a portion of which the liver is subsequently formed. Owing to the large amount of yolk in the egg, the yolk-mass projects beyond the head of the embryo, and so it happens that the diverticulum extends from the gut portion of the archenteron in an anterior as well as a ventral direction, following the curve of the yolk (see Fig. I). In stage

IV, folds are formed in the anterior or more strictly speaking dorsal wall of the diverticulum, in that portion of the diverticulum which lies nearest to the archenteron and immediately posterior to the heart. The liver develops from the folds and the lumen of the more anterior part of the diverticulum becomes obliterated. With the mesoderm covering the folds, blood-vessels are carried into the substance of the liver, and between the two deepest folds runs a large vitelline vein, which leads directly into the posterior end of the heart (see horizontal section of stage IV, Fig. G1, rl, vv). The heart through several stages, abuts against the anterior end of the liver, and as the liver differentiates, the spaces filled with blood between the trabeculæ, are in direct communication with the sinus venosus (Fig. F, 1 and sv).

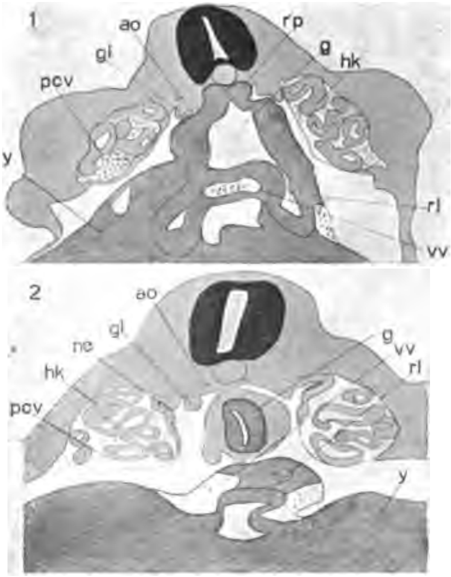


Fig. G. 1, horizontal section of stage IV; 2, cross section of stage V. ao, aorta; g, gut; gl, glomus; hk, head-kidney; ne, nephrostome; rl, rudiment of liver; r'p, 1st rudiment of pancreas; vv, vitelline vein; y, yolk-mass.

As the liver develops, it is surrounded by the splanchnopleure and is suspended from the dorsal wall of the body cavity; the portion of the gut from which the stomach develops lies to the left of the mesentery thus formed (Fig. H2, 3). The communication between the liver and gut is gradually reduced to a small opening (stage VII), the definitive bile-duct, which enters the gut close to its connection with the central yolk-mass. As the gut is lengthened at the expense of the yolk-mass, the

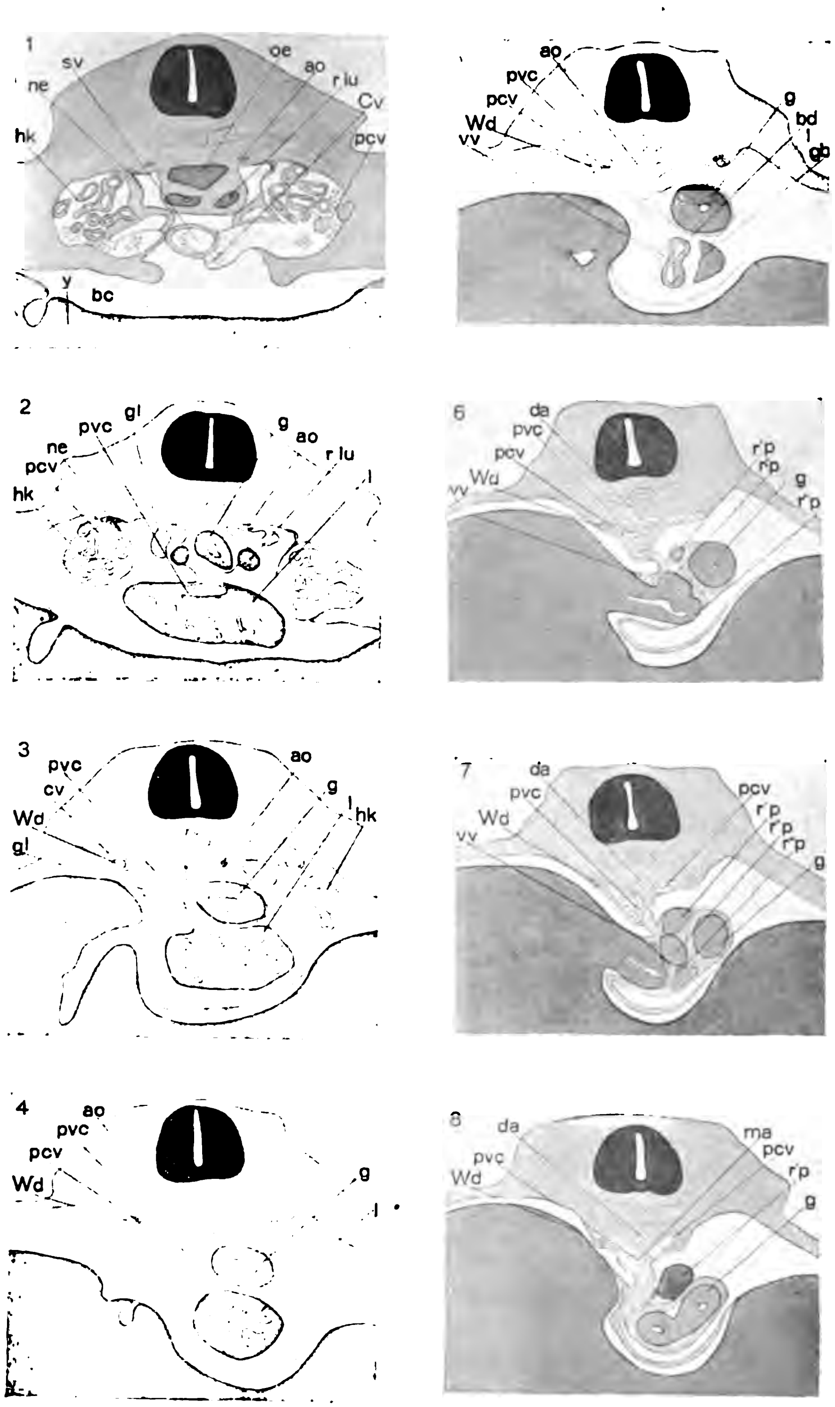


FIG. H. Cross sections of stage VIII. 1-8 from before backwards. ao, aorta; bc, body cavity; bd, bile duct; Cv, Cuverian vein; da, dorsal aorta; g, gut; gb, gall bladder; gl. glomerulus; hk, head-kidney; l, liver; ma, mesenterial artery; ne, nephrostome; oe, oesophagus; pcv, posterior cardinal vein; pvc, posterior vena cava; rlu, rudiment of the lung; r'p, r''p, r'''p, 1st, 2d, 3d rudiment of the pancreas; sv, sinus venosus; vv, vitelline vein; Wd, Wolffian duct; y, yolk-mass.

yolk recedes from the region where the liver is established (cf. Fig. F with Figs. G and H 4-8). The lengthened gut forms a loop posterior to the liver and joins the yolk-mass on the right side. The bile-duct is increased in length and in stage VIII still opens into the gut near the point where the gut joins the yolk-mass (see Fig. H5, 6); since the gut has lengthened, it seems that the entrance into it of the bile-duct has shifted toward the yolk. The gall-bladder appears about stage VI as a diverticulum from the ventral wall of the bile-duct close to the liver (Fig. F, gb. cf. Fig. H5).

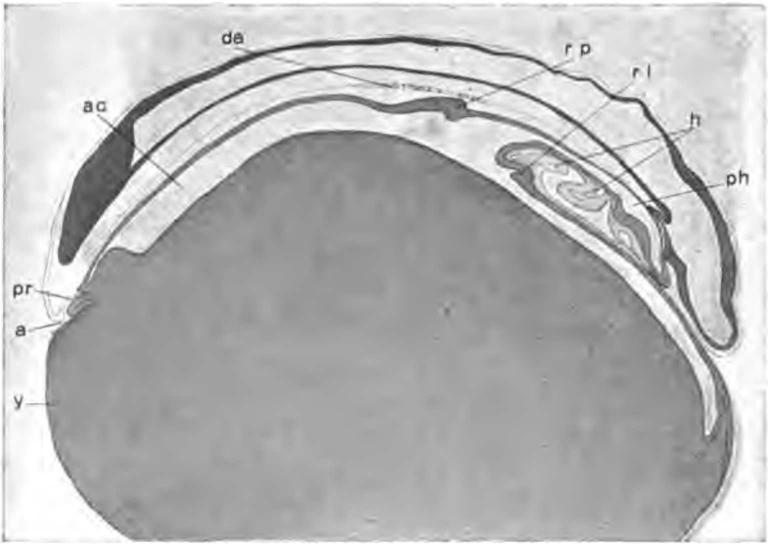


FIG. I. Sagittal section of stage IV (nearly in the median plane). a, anus; ac, archenteric cavity; da, dorsal aorta; h, heart; ph, pharynx; pr, proctodaeum; rl, rudiment of liver r′p, 1st rudiment of pancreas; y, yolk-mass.

Pancreas.—The pancreas develops from three rudiments. The first in time of appearance is present in stage IV as a diverticulum from the dorsal wall of the gut (Fig. G1, I, r′ p). Anteriorly this rudiment ends in a solid mass of cells containing much yolk, and entirely separated from the gut. The connection between the gut and this rudiment is already lost in the next stage.

Early in stage VI, a second rudiment appears as a diverticulum from the dorsal wall of the definitive bile-duct nearer to the gut than to the liver (cf. a later stage Figs. H6, 7, r″ p). The third rudiment arises later, in the same region as the second, but from the ventral wall of the duct (cf. Fig. H6, 7, r‴ p). The connection with the definitive bile-duct is retained where the second and third rudiments originate. No trace remains of the place of evagination of the first rudiment from the gut itself.

Mouth.—The stomodæum is from its first appearance (after stage IV) slit-shaped, and lies well under the ventral surface of the head (cf. Fig. 15, Plate II). A free passage from the stomodæum into the mouth cavity is not established until stage X.

A noteworthy fact in the development of the mouth is the entire absence of horny jaws, and teeth, and of fringed lips. The hardened tip of the upper lip which develops late in embryonic life will be described in another place: it has not the characteristics of a tadpole tooth. It may be here noted that an adhesive gland is also wanting. The tongue is first found in stage VII.

Visceral Pouches.—The visceral pouches first appear in the series in stage III. It has been noted that, in surface view, there are four ectodermic depressions. In section, it is found that the hyo-mandibular pouch is a deep diverticulum from the wall of the pharynx and that its entodermic lining in many cases lies in contact with the outside ectoderm; the first branchial pouch is in almost the same condition, but not in actual contact with the ectoderm; the second branchial pouch is merely a shallow diverticulum, and the third barely begun. In stage IV (Fig. J), the same conditions exist, except that the third branchial pouch has become deeper. After stage IV the pouches are gradually reduced, and before VIII have almost disappeared.

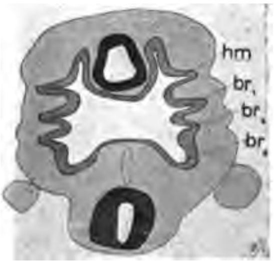


FIG. J. Horizontal section through the branchial pouches in stage IV. br 1, br 2, br 3. 1st, 2d. 3d branchial pouch; hm, hyomandibular pouch.

Not one of these pouches in any preparation can be demonstrated to open to the outside, hence the evidence as far as it goes shows that no true gill-slits are formed in the embryo of Hylodes. Before this conclusion can be placed entirely beyond doubt, it is necessary that a more complete series of embryos be examined.

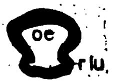


FIG. K. Cross section of the oesophagus and rudiment of the lung in stage V. oe, oesophagus; rlu, rudiment of lung.

The statements of earlier writers in regard to the gills have been entirely confirmed. Not a trace of either external or internal gills can be found in any of the embryos. It will be shown later that the circulation in the gill arches is extremely simple.

Œsophagus and Lungs.—The lungs are derived from a single rudiment which arises in stage V by a constriction of the ventral portion of the œsophagus from the dorsal (Fig. K). As the rudiment becomes more completely separated from the gut, the œsophagus is flattened dorso-ventrally, and loses its lumen (Fig. L). The solid portion of the œsophagus extends for a little distance anterior and posterior to its union with the lungs (Figs. L1, 2 and H1, œ, rlu). The lung rudiment lengthens and becomes paired posteriorly.

The foregoing description shows that by the time stage VIII is reached, the principal adult organs derived from the archenteron are well differentiated. The liver, after stage VIII, becomes more compact; it is less intimately connected with the heart, and definite efferent hepatic vessels are formed. The gall-bladder increases in size and after stage X contains a clear, yellowish substance. The three rudiments of the pancreas become unified into a compact organ. The lungs increase in size, and soon after stage VIII hang freely in the body cavity, surrounded by the splanchnopleure. Their walls and the walls of the œsophagus are somewhat pigmented after stage XII. The lungs, even as late as the time of

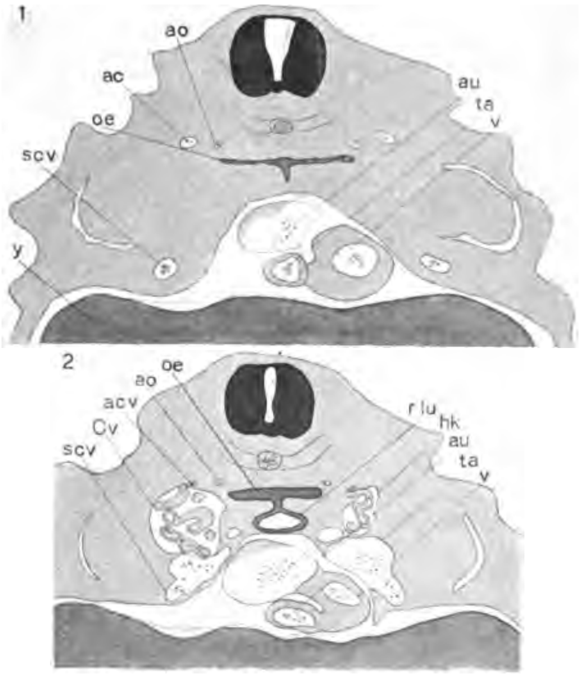


FIG. L. Cross sections of stage VIII. 1 anterior to 2 (both anterior to Fig. H1). acv, anterior cardinal vein; ao, aorta; au, auricle; Cv, Cuverian vein; hk, head-kidney; oe, oesophagus; rlu, rudiment of lung; scv, subclavian vein; ta, truncus arteriosus; v, ventricle; y, yolk-mass.

hatching, have attained no great size and are remarkably simple in structure. In sections of the late stages, and in adults preserved in alcohol, they appear as hollow thin-walled sacs, not showing the trabecular structure of the lung of the common frog. In all sections of late embryonic stages, the walls of the lungs are more or less wrinkled, so that in life they may have been capable of expansion to some extent; in their present condition, however, they extend, at most, only slightly beyond the anterior limit of the liver.

Differentiation of the alimentary canal, after stage VIII, is accompanied by the disappearance of yolk from its walls, and by the absorption of the large yolk-mass that still separates the anterior part of the intestine from the posterior (cf. Figs. B1, and O for the size of the yolk relative to the embryo). The yolk first disappears from the walls of the pharynx. In closed portions of the gut, the lumen as a rule reappears immediately upon absorption of the yolk, but the roof and floor of the broad low œsophagus remain pressed together, even after there is no yolk remaining in them, and the opening to the lungs is not re-established until after stage XII. The stomodæum is open into the entodermal buccal cavity at stage X (as already stated), so that after XII, there is free communication from the lungs to the outside. Posterior to the lungs, the walls of the œsophagus, after the reduction of the yolk in their cells (stage XII), become convoluted but no lumen exists until after hatching. The convolutions may be partly due to shrinkage in the preparations, although in an older specimen where food has entered the stomach, the walls remain distended after preservation; this seems to indicate that the walls remain collapsed until food has passed through the gut. The stomach portion of the gut retains its lumen from the time of its formation. The intestinal portion continues to be lengthened after stage VII by additions from the yolk, has a small lumen, and becomes convoluted about stage XII. The walls of the posterior gut lose their yolk first in the cloaca, then in the portion near the yolk, and lastly between these parts. As in the anterior gut, the walls become convoluted in stage XII. The lumen near the middle yolk is enlarged in stage XII and extends through the gut for some distance posterior to the yolk.

Since the posterior gut is constantly added to from one side (the left) of the yolk, and the anterior from the other side, it comes about that the two connections between gut and yolk pass each other, and a middle region between them does not exist after stage XII in the same sense as before. Still there remains a large amount of yolk, which after stage XI does not retain its solid condition (see Fig. M), and is no longer reduced by gradual contributions to the more differentiated parts of the gut.

In stages XI to XII, the interior of the yolk is split in various places, and the fissures contain a substance which stains deeply with carmine. The substance may be the product of cells that appear to be breaking down in the borders of the fissures. In the next stage (XIII), the fissures have formed a continuous cavity that leads into the anteriorly and posteriorly differentiated gut, thus completing the lumen of the alimentary canal. In the meantime, the whole yolk becomes lobed, and large portions are contributed to the gut at once. This takes place with almost uniform regularity; the first lobe is formed in stage XII, and from that part of the yolk which immediately joins the anterior gut; the second is added from the yolk in connection with the posterior gut. The amount of yolk is constantly reduced in the large lobes, and finally in stage XIV the yolk is found in only a small portion of the intestine (cf. Fig. M).

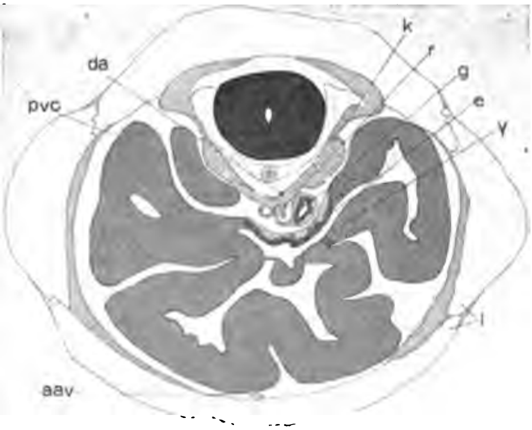


Fig. M. Cross section of stage XIV. aav, anterior abdominal vein; da, dorsal aorta; e, differentiated portion of entoderm; g, gut; k, kidney; ls, lymph spaces; pvc, posterior vena cava; r, reproductive organ; y, entodermic cells laden with yolk.

A comparison of Figs. B1, 2, 3, Fig. M, Fig. C and Figs. I, F, O, will show briefly, in review, the main facts in the history of the yolk-mass and its relation to the embryo.

There is no coiled intestine as in the vegetable feeding larva of Rana, but the yolk holds the same relation in the digestive tract as it does in two other yolk-laden Amphibian eggs, those of Plethodon cinereus and Ichthyophis glutinosus (cf. Fig. B3 with Montgomery '01, Fig. 5 and Sarasin '87, Fig. 7, and cf. Fig. N with Sarasin '87, Fig. 8). Very much the same relations appear from the descriptions to exist in Alytes obstetricans at one period, although in this form a coiled intestine is later developed.

From the earliest stages of the series, the entire yolk is divided into cells, which are larger in the center and smaller at the periphery (Fig. F). The cell walls are sometimes not clear in poorly preserved eggs of early stages.[4] The cells are, in all stages, smallest in that part of the yolk where the anterior and posterior parts of the gut are forming. In later stages while the yolk is disappearing from the gut walls, it is lost also in the cells of the middle yolk-mass immediately beneath the mesentery and by the time the cavity of the alimentary canal is established in the central yolk-mass, the wall beneath the mesentery is differentiated into a layer of small cells that pass abruptly into huge yolk-laden cells several times their own size (Fig. M, g, e, y).

The frog may feed before the yolk is entirely absorbed, for the stomach of a hatched frog contains green food, while part of the intestine is still loaded with yolk.[5]

3. HEART AND BLOOD-VESSELS.

The details in the development of the blood-vessels have not been worked out, but the chief events in their history have been determined.

Heart.—The heart develops from the mesoderm ventral to the pharynx.

In stage IV, it has arrived at the condition of a bent tube (Fig. I) with a slight constriction between the auricular and the ventricular parts. It undergoes the usual changes of further twisting, thickening of the ventricular walls, etc. The longitudinal septum in the truncus appears at about stage VII, but the septum between the auricles, which in the frog appears simultaneously with the septum of the truncus, is not found in Hylodes until stage IX; it attains no great size until XI, after which it separates a very small left auricle from the right. The relatively late development of this septum may be correlated with the slow growth of the lungs and consequently of the pulmonary circulation (cf. p. 493).

Veins.—For a full understanding of the vitelline circulation, the examination of living material is essential. From the preserved preparations no constant arrangement of vessels on the yolk can be determined. In stage IV, the yolk is literally covered with great vessels, in which are large blood corpuscles filled with yolk granules; the vitelline blood is collected in a large vein, which passes through the liver, and constitutes in early stages the posterior end of the heart (Fig. G1, vv). After stage VI, the vitelline vein comes from the right side of the yolk (where the liver, yolk and gut are connected), passes along the dorsal wall of the liver, and joins the posterior vena cava before it enters the heart. Lastly after about stage VIII, the vitelline vein (Fig. H7-4, vv) is greatly reduced and no longer passes through the liver as a distinct vessel, but is lost in the tissues of the organ.

---

[4] It is stated that the yolk-mass of Desmognathus fusca and of Ichthyophis is at one period segmented only peripherally, and that in Plethoden cinereus also the boundaries of the yolk-cells disappear.

[5] The embryo of a viviparous salamander will feed although yolk is still contained in the digestive tract.

The Cuverian veins, the anterior cardinals, and perhaps the posterior cardinals are present in stage IV (cf. Fig. N, with the following description). There is also, on each side of the yolk, an enormous cutaneous vein, which lies in the somatopleure, hence not directly in contact with the yolk-mass, and passes beneath the forelegs into the Cuverian vein. It is so conspicuous from stages VI to IX, that it deserves especial mention as an embryonic vessel (see Fig. L1, 2, scv). From its position the cutaneous vein would seem to be the forerunner of the subclavian vein, but it has not been seen after stage XII.

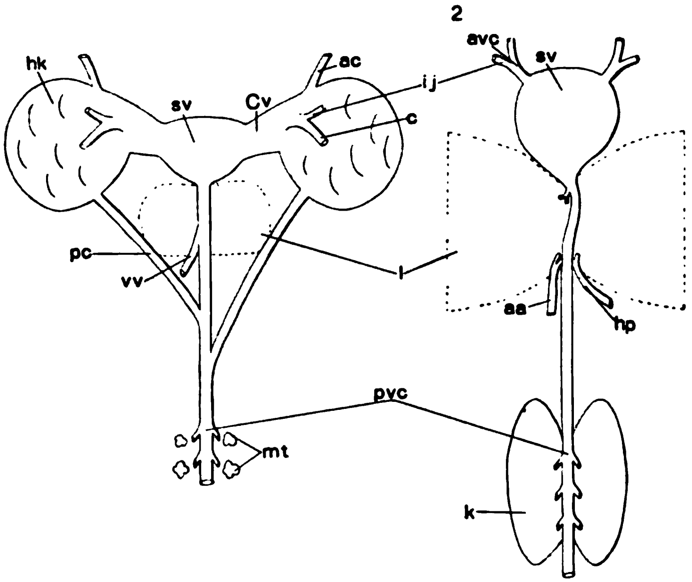


FIG. N. Diagram of veins. 1, stage VIII. 2, stage XV. aa, anterior abdominal vein; ac, anterior cardinal vein; avc, anterior vena cava; c, cutaneous vein; Cv, Cuverian vein; hk, head-kidney; hp, hepatic portal vein; ij, inferior jugular vein; k, kidney; l, liver; mt, mesonephric tubule; pc, posterior cardinal vein; pvc, posterior vena cava; sc, subclavian vein; sv, sinus venosus; vv, vitelline vein.

The posterior cardinals are evident after stage V. They diverge from a common trunk (2 sections posterior to Fig. H8), and passing anteriorly, bathe the head-kidneys and empty into the Cuverian veins (Fig. H8-1, pcv, cv). The posterior cardinals are remarkably conspicuous from stages VI to IX, and persist to stage XII, when only vestiges of them can be found posterior to the degenerated head-kidneys.

The anterior cardinals (Fig. L, acv) also empty into the Cuverian veins. After the degeneration of the posterior cardinals, the Cuverian veins and anterior cardinals persist as the anterior venæ cavæ. After stage VII, the inferior jugular veins from the ventral region of the neck enter the Cuverian veins with the cutaneous, and persist after the cutaneous veins have been reduced. In stage XV, they are present as branches of the anterior venæ cavæ.

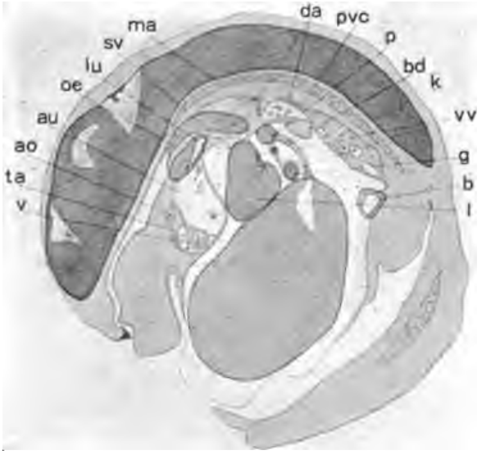


FIG. O. Sagittal section of stage XII (drawn to same scale as Fig. B1). ao, aorta; au, auricle; b, bladder; bd, bile duct; da, dorsal aorta; g, gut; k, kidney; l, liver; lu, lung; ma, mesenterial artery; oe, oesophagus; p, pancreas; pvc, posterior vena cava; sv, sinus venosus; ta, truncus arteriosus; v, ventricle; vv, vitelline vein.

The posterior vena cava appears at about stage VI, as a median vein, which passes along the dorsal wall of the liver, receives the vitelline vein, and empties into the sinus venosus (cf. Fig. H7-2). As the vitelline vein becomes less prominent the vena cava increases in importance, and takes its place as the posterior end of the sinus venosus. In later stages, as the heart becomes separated from the liver, the posterior vena cava is also separated from the liver (Fig. O) and receives definite efferent vessels from it. The posterior vena cava joins the right posterior cardinal (Fig. H6, 7, pvc, pcv) at a little distance anterior to the union of the two cardinals; and after the posterior cardinals disappear, continues to hold the same course. As the posterior vena cava, where it joins the heart, acquires greater importance than the vitelline veins, and replaces the posterior cardinals in the trunk, it becomes the principal vein posterior to the heart. It receives numerous branches from Wolffian bodies, and is the vein of the very vascular tail.

The hepatic portal vein cannot be distinguished before stage IX. The vitelline vein joins it at the liver, and is its most conspicuous branch until stage XII.

The anterior abdominal vein has been first observed in stage XIII. It runs in the median line (cf. Fig. M, aav), from the posterior end of the embryo, along the ventral surface of what remains of the yolk, bends back around the anterior limit of the yolk, passes posteriorly for a short distance in the pericardium and enters the liver with the hepatic portal. The stages are wanting to determine whether the anterior abdominal has a paired origin, and whether when it first develops, it opens directly into the sinus venosus. In stages XIV and XV, when the yolk has gone from the region of the heart and liver, the anterior abdominal passes directly from the ventral wall of the abdomen into the liver, making no loop anteriorly as in stage XIII. The vesical vein, a branch of the anterior abdominal from the bladder, is found in stage XIV.

Arteries.—The dorsal aorta in stage IV is fully established; it is formed by the union of the aortic arches and extends to the posterior end of the embryo. It might fairly be expected that so large a quantity of yolk as the embryo possesses would be supplied by a fixed arterial system, but it has been impossible to trace any definite arteries to the yolk-mass at this stage. It can only be said that the infolding walls of the posterior gut are surrounded by blood corpuscles, and there is some evidence that a branch from the aorta connects with this region.

In stage VI, a branch of some size passes from the aorta into the mesentery which slings the yolk of the middle region (Fig. F, B3, ma); thence it runs posteriorly along the rod of yolk, and posterior gut (Fig. E, ma), and probably gives off branches on either side, to the yolk. The vessel, reduced in size, persists in later stages as the cœliaco-mesenteric artery (Figs. H8, O, ma). The point where it leaves the dorsal aorta is slightly posterior to the union of the two aortæ, and in the region between the union of the posterior vena cava with the right and with the left posterior cardinal. The point of union with the left cardinal is, as it were, thrown back to admit of the passage in the mesentery of this little branch of the aorta. The arterial system in the visceral arches has been worked out as far as possible in the present series; but it is hoped, as in the case of the visceral pouches, that the observations may be amplified by the study of more abundant material. The vessels of the arches are characterized by extreme simplicity. The first vessel to be completed is the aortic arch of the first branchial arch in stage IV; very soon the vessel of the second arch is also complete. In the meantime incomplete vessels or lacunæ are found in the mandibular and hyoid arches, but at no time is there a complete vessel from the truncus to the collecting aorta in any visceral arch save in the first and the second branchial.

The pulmo-cutaneous vessel when first seen in the series, at about stage V to VI, branches near the truncus from the aorta of the second branchial arch. A study of fairly abundant material shows no indication of the origin of the pulmo-cutaneous from an artery of the fourth branchial arch, as in the frog. There is no representative of the vessel of the third branchial arch.

The condition then in stage VI (Fig. P) is as follows:—The first branchial arch passes from the truncus into the collecting aorta, and is continued anteriorly as the carotid. The aorta of the second branchial arch passes posteriorly as the systemic, meeting the corresponding vessel of the other side, posterior to where each has supplied the glomus of the head-kidney. The collecting aorta is already somewhat reduced between the dorsal parts of the first two arches. Lacunæ are still found in the mandibular and hyoid arches; the vessel in the hyoid is connected with the ventral portion of the aorta of the first branchial, but the aorta is not complete in either mandibular or hyoid arch. Finally, at the point where the first and second branchial arteries separate, a more posterior branch, the pulmo-cutaneous, is given off.

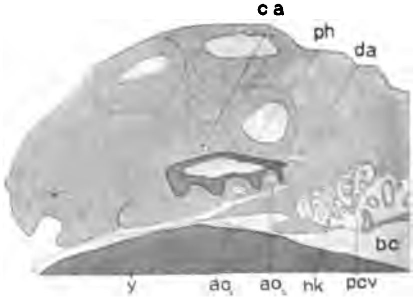


FIG. P. Sagittal section of the head in stage VI. ao_1, ao_2, aorta of the 1st and of the 2d branchial arch; bc, body cavity; ca, carotid artery; da, systemic aorta; hk, head-kidney; pcv, posterior cardinal vein; y, yolk-mass.

In stages soon after VI, as the visceral arches are obscured, the collecting aorta between the carotid and systemic degenerates, and the embryonic arrangement of the aortic arches cannot be detected; the carotid, systemic and pulmo-cutaneous arise as three great branches from the truncus.

Pulmonary circulation.—The study of the pumonary vessels has given mostly negative results. The cutaneous artery is an established vessel from stage VI on, but in the same section where it can be clearly traced, nothing can be seen of the pulmonary artery in specimens younger than the stage of hatching. In a longitudinal section of stage XV, it appears as a branch of the cutaneous vessel. The pulmonary vein has not been seen with certainty in any stage. As other vessels are clearly seen in the preparation, it follows with great probability that the pulmonary vessels are very small or wanting in embryonic stages. It will be remembered that the lungs are small, and also that the left auricle is very small, and the septum between it and the right auricle is late in developing.[6] All the facts then in connection with the morphology of the organs for pulmonary respiration, go to show that the lungs are not of great functional importance in embryonic stages.

Lymph sinuses.—Sub-dermal lymph sinuses appear about stage X as narrow spaces; by stage XIII they are enlarged into enormous sacs that completely surround the animal (cf. Fig. M). They are separated by narrow partitions, the principal sinuses being a dorsal, a ventral, and lateral chambers.

4. EXCRETORY AND REPRODUCTIVE ORGANS.

The excretory system of Hylodes has been described by Selenka, 82, from a reconstruction of one stage (about VI, judging from the figure independently of the excretory system). He has added notes on a later stage (about XIII). Selenka concludes that the pronephros degenerates and the mesonepronephros appears early as compared with other amphibia, and he associates this condition with the absence of gills, a combination approaching the condition in Reptiles. A comparison of this sort requires further evidence from the study of the function of the organs.

Field, 91, interprets Selenka's figures and description as showing that the pronephros in the earlier stage appears already in a degenerate condition, the collecting trunk being extended into irregular blind sacs instead of convoluted, and opening directly into the anterior end of the segmental duct. I have not made reconstructions of any stage, so that I cannot judge of the accuracy of Selenka's[7] figures of the model, but in section the tubules appear to be, through several stages including Selenka's early stage, as regular as in sections of Rana. It would be of interest to determine the exact state of the organ from a series of models; but at present, I can only give a brief statement of the time of appearance and duration of the principal parts of the excretory system.

---

[6] In some of the lungless salamanders the auricle is small, the septum between the auricles is incomplete, and the pulmonary vein is wanting.

[7] The spinal cord in Salenka's cross section is in the shape of a cross, which does not appear in my sections. His "J," called in the explanation of the figure "Darm," is in the usual position of the heart, while the unlettered space in the shading above it might well be the gut and rudiment of lungs. Selenka mentions the lungs as present in the older embryo but wanting in this. We have seen that the lung diverticulum is present even before VI. I have found the degenerate glomus and rudiments of the ciliated funnels in stages later than those in which Selenka mentions them.

The Wolffian ducts are found in stage II. While the archenteric cavity is still broad, their posterior ends are far apart, but when the gut has been differentiated from the archenteron, they meet in the median line, where they open into the gut. It has already been noted that from this point backward, the gut remains open as the cloaca. It seems to be the enlargement of the gut which Selenka calls the "allantois."

The tubules of the pronephros are already present in stage III in a simple form; in IV, they are much coiled, and before stage VI they form a compact organ. In stage III, the three ciliated funnels open into the peritoneal cavity (cf. Figs. G2, H1, 2, ne), the two anterior funnels close together. In XI, the tubules of the head-kidney are irregularly dilated; in XIII, the organ is reduced in size, and the tubules are solid in part. Vestiges of the ciliated funnels persist until XIV, when their number cannot be determined from the sections. In XV, the degenerate pronephros has almost entirely disappeared. The Wolffian duct remains connected with the head-kidney until stage XV, when only a vestige of it remains anterior to the Wolffian body. The glomus is present in stage IV (Figs. G1, 2, gl), and develops and degenerates at a nearly equal rate with the pronephros, except in later stages, when it is particularly large relatively to the size of the head-kidney. In stage XV a vestige of it still persists.

About four or more mesonephric tubules are present in stage VI, and from that time they increase in number, size and complexity until they have become a compact mass (stage XV, cf. Figs. M, O, k).

The bladder appears at about stage X as a solid projection of the ventral wall of the cloaca. As it develops, it extends anteriorly, becomes hollow and distally bifid (from stage XI), and by stage XIV, it is a thin-walled cleft sac of considerable size (cf. Fig. O, b).

The genital ridges arise at about stage VII, and are well developed in stage XV (cf. Fig. M, r). At that time, there is no appearance of degeneration of the anterior portion into fat bodies, no differentiation into testes or ovaries, nor has the rudiment of the Müllerian duct been seen.

5. TAIL.

The tail of Hylodes calls for particular attention because of the unusual condition of almost every feature of it. In a word, it constitutes not a muscular organ for locomotion but a vascular organ for breathing.

The notochord is the axis of the tail; the flat surfaces are morphologically the right and left sides. Thus the vascular lamellæ of the tail are in the position of the dorsal and ventral fins of the tadpole. To whichever side the tail is turned in the egg the part which lies cephalward from the notochord is dorsal, and the part on the opposite side of the notochord is ventral. These relations are clearly established by the relative positions of the notochord, spinal cord, and dorsal aorta (Fig. Q). While the tail after stage IV becomes broader and thinner, as described from the surface view, the connective tissue becomes spongy, and through its meshes is a network of capillary blood-vessels. The ventral part is more richly supplied with blood-vessels than the dorsal, a condition perhaps correlated with the fact that it contains the dorsal aorta which carries the main supply of blood. The aorta extends with the notochord nearly to the end of the tail; no main efferent vessel runs for any distance through the tail, but the blood is collected in the posterior vena cava near the union of the tail with the body.

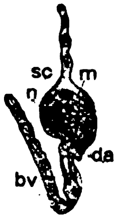


FIG. Q. Cross section of the tail in stage VIII. bv, blood-vessels: da, dorsal aorta; m, muscle: n, notochord; sc, spinal cord.

At the base of the tail the diameter of the notochord from stages VI to XII far exceeds its diameter in the body of the embryo. In the tail itself the notochord diminishes in size toward its distal end. The diameter of the spinal chord is greatly reduced as compared with its size in the body, especially in late stages. Surrounding the notochord is a layer of muscle that is connected with the muscles of the body and like them is segmented. No vertebræ are found in the tail.

In stage XIV the notochord, muscles and spinal cord have become reduced, and the tail is more solid but still very vascular; in stage XV when the tail is almost entirely absorbed the tissue is compact.

6. DERMAL FOLD.

The fold of skin, which in several embryonic stages partially covers the arms, will be described in more detail. The anterior leg lies in a space which is the angle between the head of the embryo and the yolk (see figure). Across this space stretches the dermal fold whose free anterior edge reaches from the head to the side of the yolk. The fold is composed of two layers of ectoderm separated by mesoderm. The folds of the two sides are entirely independent of one another. The fold differs from the operculum of the tadpole, in that it is continuous with the epidermis of the body posteriorly, and its free edge is directed anteriorly. But the anterior legs of Hylodes, for some time after their first appearance, are directed anteriorly from the body because of the large amount of yolk at the sides of the embryo; hence the dermal fold resembles an early stage of the operculum of the tadpole in its morphological relation to the anterior leg.

The entire separateness of the dermal folds of the two sides recalls the condition of the operculum in the two representatives of the Aglossa, Dactylethra and Pipa, where the operculum opens on both sides. In these two forms, however, the anterior legs are not covered by the operculum.

It is not evident from the points of difference and of resemblance between the dermal fold of Hylodes and the operculum of the tadpole whether or not the two structures are homologous.

BEAK.

The black tip on the upper lip is seen at late stages in external views (Plate II). It is triangular in sagittal sections (Fig. O), appearing in sections at about stage X. The beak does not surmount a column of epithelial cells like the teeth and edge of the jaw of the tadpole. It attains its greatest size in the last stage within the egg, and whether by accident or normally, the tip is sharp in stages XI, XII and XIII, and blunt in the hatched tadpole. The facts suggest that its function is to rupture the egg membrane (cf. Rana opisthodon).

SENSE ORGANS.

I have not attempted to make a study of the sense organs, but observe that they arise in very early stages as in the tadpole.

The auditory vesicles are already seen in stage II. In the same stage, the optic vesicles are in the condition of diverticula from the brain; their connection with the brain is reduced in the next stages, and in III the lenses are being formed. Thickenings of the epidermis, the rudiments of the anterior nares are present in stage III, and the hollow diverticula, reaching anteriorly from the pharynx, are, to all appearances, the beginnings of the posterior nares.

7. COMPARISON WITH OTHER FORMS.

In immature stages of Amphibia, there are various kinds of adaptations to different surroundings. In each of the three living orders, there are exceptions to the rule that the Amphibia develop from eggs laid in water and pass through a free-swimming stage. Among the Urodeles are the viviparous Salamandra atra, also Salamandra maculosa, and the forms with terrestrial eggs (some species of Amphiuma, Plethodon cenereus Antodax lugubris, Desmognathus fusca); among the Gymnophiona are Typhlonectes compressicauda, and Dermophis thomensis, two viviparous species, Hypogeophis, in which the eggs are terrestrial and the larval life is completed within the egg-membranes, and Ichthyophis glutinosus, with early stages out of the water; and among the Anura are a number of forms recently enumerated by several writers.[8]

The facts as far as they are known in regard to the development of the exceptional forms point to the interpretation that they are descended from forms which originally had aquatic larvæ.

It is to be noted that among Anura, there are no known cases of internal fertilization and viviparity.[9]

It seems clear, from what has been described of the habitats, that a form with a free-swimming larva would not survive in some at least of the regions where the animals are found. Salamandra atra lives in high Alps, where the water runs swiftly. Hylodes and Rana opisthodon also live where there are no pools. In the latter cases, the larva and adult no longer inhabit two different environments, but the embryo has become adapted to the environment of the adult of the original form.

There are other ways in which the difficulties of insufficient water are met. Development may be hurried through (in Lythodytes latrans) in a rain pool, where the supply of water is uncertain, or the tadpoles may be provided for in such a way as to survive a drought, as in Cystignathus mystaceus. In such a case as that of Pipa, in which the eggs are carried on the back, while the parent stays in the water, the advantage of the habit is not obvious at present.

The various adaptations involve in different degrees not only the larvæ but often the instincts and certain structures of the parents (as when pouches are developed).

Whatever the cause or origin of the adaptations may be, it is found that, with a few exceptions, the larvæ or embryos of all the forms still retain some of the organs characteristic of the free-swimming larva. These organs are not, however, always used in the same way, or even for the same purpose as in the aquatic larva. In some cases (and among them Hylodes) all the larval organs are not present, and furthermore some new organs which are not found in the larva occur in the embryonic forms.

The most patent differences in the requirements of the aquatic larva and of the terrestrial or viviparous embryo concern nutrition and respiration. In the embryonic form there is the necessity for a special food supply and for organs of respiration adapted to a medium other than water.

---

[8] Brandes and Schoenichen, 01. Wiedersheim, 00. Sampson, 00.

[9] In Pipa, fertilization takes place perhaps in the oviduct, which is curiously protruded over the back of the female when the eggs are laid.

In the viviparous salamander, the larva imbibes the fluid within the uterus in which it swims. In terrestrial forms of each order, the food supply is given to the embryo in the enormous mass of yolk in the egg. In spite of the large size of the egg, the type of cleavage in Hylodes is holoblastic as in other Anura. The yolk-laden eggs of other forms have scarcely been studied. The holoblastic type has been found to occur in Alytes and in Desmognathus fusca (both formerly supposed to have meroblastic eggs).

As would be expected, the number of the eggs is comparatively small in forms where the eggs are excessively large. The absence of pigment is another peculiarity that has been noted in many cases where the eggs are concealed, either carried by the parent or laid in sheltered situations. The following table summarizes the observations, so far as they have been made:

	Color of Eggs.	Deposition of Eggs.	Number of Eggs.	Size of Eggs.
Nototrema marsupiatum.		Dorsal pouch ♀.	200	"Smaller than a pea."
Nototrema oviferum.	Not pigmented.	Dorsal pouch ♀.	15–30	1 cm.
Nototrema fissipes.		Dorsal pouch ♀.	16	10 mm.
Nototrema pygmaeum.		Dorsal pouch ♀.	4–7	
Pipa Americana.		Back of ♀ in the water.	100	
Rhinoderma darwinii.		Gular pouch ♂.	10	
Rhacophorus reticulatus.		On abdomen ♀.	21	
Hyla goeldii.	Not pigmented.	Back of ♀.	10–26	4 mm.
Hylodes martinicensis.	Not pigmented.	Under stones, etc.	30	3–4 mm.
Phyllomedusa jheringii.		On trees.	200	2 mm.
Alytes obstetricans.	Not pigmented.	Round legs of ♂.	18–54	3½–5 mm.
Cystignathus mystaceus.	Not pigmented.	Nest under stones, etc.		
Rhacophorus schlegelii.	Not pigmented.	Nest in the side of a bank.		1 mm.
Polypedates maculatus.	Not pigmented.	Sides of cisterns, etc.		
Paludicola fuscomaculata.	Not pigmented.[10]	Floating in small pool.		1 mm.

---

[10] A case of unpigmented eggs laid in small pools; these eggs are not long exposed, for they hatch eighteen to twenty-four hours after the beginning of segmentation.

The development of the digestive tract has heretofore scarcely been touched upon in the descriptions of the yolk-laden eggs. We have seen a close similarity in the history of the yolk-mass in Hylodes and in the few forms already studied, and the absence in Hylodes of a coiled intestine.

In the embryo of Hylodes, fed by the yolk in the egg; of Rhinoderma darwinii, developed in the gular sac of the male; of Pipa americana, on the back of the female; of Nototrema oviferum, in the dorsal pouch of the female, no horny teeth or jaws have been found. (The presence or absence of teeth is not recorded in the case of other protected embryos.) It is also to be noted that no adhesive gland is found in Hylodes.

Passing now to the organs of respiration, we find that different parts of the body serve for breathing in different forms, and that there is great diversity in the number and form of the gills, where present.

In the viviparous salamander, the gills are finely feathered, and serve for breathing in the fluid of the uterus. Larvæ put into water do not continue to use their gills unaltered; the gills either undergo certain changes or are lost, and new gills or other organs develop. In Alytes, the eggs are carried for a time on the hind legs of the male, and the external gills are represented by one long branched pair. In Pipa americana, in which the eggs are carried in water by the parent, three pair of external gills develop, but these are lost early and the internal gills appear. In Nototrema oviferum, in which the eggs are carried in the dorsal pouch, one pair of bell-shaped gills occur, which seem to represent two fused gills on each side. They are present before the tadpole leaves the maternal pouch, and also for a short time afterwards. A gill rudiment on the third arch may represent the internal gills, but no others are present. In Nototrema marsupiatum, it is said that there is no trace of external gills in the embryo when it leaves the parent's pouch. Finally, the gills are entirely wanting in Hyla göldii, whose eggs are carried on the back of the female; in Rana opisthodon, whose eggs are found in crevices of rocks; in Rhinoderma darwinii, whose embryos are contained in the gular pouch of the male; and in Hylodes martinicensis, whose eggs develop on land. In Hylodes even the gill-slits are probably wanting.

Respiration is carried on by some other means, which in some of these cases has not been recorded. In Hylodes we have seen that the tail is adapted to this function, i. e., the new function of breathing in air has been assumed by an ancestral larval organ no longer useful in the original way. In Rana opisthodon, the tail is not present in advanced embryos, and numerous folds on the sides of the embryo probably function as respiratory organs.[11]

In other forms besides Hylodes, the tail differs from the swimming-tail of the tadpole. In Nototrema marsupiatum it is said to be vascular; in the tadpoles of Hyla abbreviatus, which slip about (even before their legs appear) on moist perpendicular rocks, the ventral fin is flattened, and seems to serve as a sucker; in Rhinoderma darwinii the tail is small and weak. In Hypogeophis and in Autodax, the fin is wanting.

Lastly, in Hylodes and in Rana opisthodon the little median horny tip in the upper lip is found in a late embryonic stage, and is used in the latter, at least to rupture the membranes in which the embryo is so long encased.

In conclusion, we find that the Amphibia are a plastic group, and among Anura especially there are patent examples of the adaptation of the larval stages to new external conditions, while the original plan of structure of the adults has remained unchanged. There are also other forms known in which, although the adults are very similar, the embryos have different modes of development. In such cases we must suppose that the embryos have diverged in their development while the end-result of their development, namely, the adult form, has remained virtually unchanged. For example, there are two species of Peripatus very similar in adult stages, but which arrive at the adult form through dissimilar embryonic stages. There are also various species of Alpheus and of Palaemonetes that have very similar adults, but whose embryonic metamorphosis differs according to the degree of abbreviation of the development of the different species.

Furthermore as Wilson has pointed out in this connection, it has been shown in the Ascidians that the same adult form may be produced by methods as different as budding and development from the egg. Finally, it is well established that in certain animals mutilation, or even removal of parts of the egg, does not prevent the development of a normal embryo.

SUMMARY.

Comparing the development of Hylodes with the development of frogs having a tadpole stage, the following points of resemblance and of difference are apparent:

---

[11] We find not only in embryos but also in adult amphibia, great diversity in breathing; respiration may be carried on by the mouth, the skin and even, according to Ritter and Miller, by vascular toes in Antodax, Plethodon and Brachoceps.

Resemblances.

1. Holoblastic segmentation.
2. The mode of formation of the blastopore, the presence of the yolk plug, and its later withdrawal.
3. The mode of development of the liver, pancreas and lungs.
4. Development of the pronephros (with its three funnels) of the Wolffian duct, and of the mesonephros.
5. The presence of a tail.

Differences.

1. A solid medullary plate.
2. In the alimentary canal:
    (a) Absence of horny jaws and teeth.
    (b) Probable absence of perforated gill-slits.
    (c) Absence of external and internal gills.
    (d) Absence of coiled intestine.
    (e) Extensive closure of the middle region of the archenteron.
3. Simplification of the circulation in the gill region.
4. Early and simultaneous appearance of all four legs.
5. Incomplete development of the lungs and of the pulmonary circulation at the time of hatching as compared with the tadpole when it leaves the water.
6. Forelegs are never completely enclosed by an operculum.
7. Changes in the tail from an organ of locomotion to one of respiration.
8. Presence of a hardened tip on the upper lip.
9. Absence of adhesive glands.

On the assumption that Hylodes has had an ancestor in which a tadpole stage existed, the development has been altered by losses, by additions, and by changes in organs already present. The structures that have been lost are: the horny jaws and teeth, adhesive glands, the perforated gill-slits, the external and internal gills, the coiled intestine, and certain blood-vessels of the gill region and of the lungs. There have been added the hard tip of the upper lip, and the early development of the legs. The tail has changed from a muscular to a vascular organ.

GERMANTOWN, PA., U. S. A., February, 1904.

REFERENCES.

Andrews, E. A., 92.—Notes on the Fauna of Jamaica. Johns Hopkins Univ. Circ., Vol. XI, p. 75.

Bavay, M., 73a.—Note sur les embryons de l'Hylodes martincensis. Extrait d'une lettre. Compt. Rend., T. LXXVII, p. 788.

—— 73b.—Rev. des sci. nat., T. I, No. 3, p. 285.

—— 73c.—Extrait de '73b. Journ. de Zool., T. II, p. 13.

—— 73d.—Note sur l'Hylodes martinicensis et ses metamorphoses. (Extrait.) Note publiée à Basse-Terre Guadeloupe en 1872. Ann. des sci. nat. Zool., T. XVII, art. 16.

—— 73e.—Note by Meyer in Zool. Garten., Jhrg. XIV, p. 356.

—— 71.—Bello Y Espinosa. Zool. Notizen aus Puerto Rico. Nach dem Spanischen frei bearbeitet von Herrn E. von Martens in Berlin. Zool. Garten. Jhrg. XII, p. 351.

Boulenger, G. A., 98.—The Tailless Batrachians of Europe.

Brehm, 92.—Tierleben. 3 Aufl.

Brauer, A., 99.—Beiträge zur Kenntniss der Entwickel. u. Anat. der Gymnophionen. II. Die Entwickel. der äussern Form. Zool. Jahrb., XII Bd., p. 477.

Bronn, H. G., 78.—Klassen u. Ordnungen des Tierreiches. Wirbeltiere, Bd. VI.

Chauvin, Marie v., 77.—Ueber die Anpassungsvermögen der Larven von Salamandra atra. Zeitschr. f wiss. Zool., Bd. XXIX, p. 324.

Cope, E. D., 85.—Batrachia. Standard Nat. Hist., Vol. III, p. 322.

Field, H. H., 91.—The Development of the Pronephros and Segmental Duct in Amphibia. Bull. Mus. Comp. Zool., Vol. XXI, No. 5, p. 201.

Hay, O. P., 88.—Observations on Amphiuma and its Young. Am. Nat., Vol. 22, p. 315.

Hopkins, G. S., 96.—The Heart of some Lungless Salamanders. Am. Nat., Vol. 30, p. 829.

Kammerer, P., 04.—Beitrag zur Erkentniss der Verwandtschaftsverhältnisse von Salamandra atra u. maculosa. Arch. f. Entwickelungsmechanik d. Organismen. 17 Bd., 2 u. 3 Hft., p. 165.

King, H. D., 03.—The Formation of the Notochord in the Amphibia. Biol. Bull., Vol. IV, No. 6, p. 287.

Marshall, A. M., 93.—Vertebrate Embryology. 1893.

Montgomery, T. H., Jr., 01.—Peculiarities of the terrestrial larva of the Urodelous Batrachian, Plethodon cinereus (Green). Proc. Acad. Nat. Sci. Phila., 1901, p. 503.

"Nature," 77.—The Development of Batrachians without Metamorphosis. Vol. XV, p. 491.

Peters, W. C. H., 75.—Ueber die Entwickelung der Coecilien. Monatsber. Akad. Berlin, 1875, p. 483.

—— 76.—Entwicklung eines Batrachiers, Hylodes martinicensis Dum, -Bibr, ohne Metamorphose. Berliner Monatsber., 1876, p. 703.

Ritter, W. E., and Miller, L., 99.—A Contribution to the Life History of Antodax lugubris Hallow, a Californian Salamander. Am. Nat., Vol. XXXIII, p. 691.

SARASIN, P. u. F., 90.—Ergebnisse naturwiss. Forschungen auf Ceylon in den Jahren 1884-85. Bd. II, Wiesbaden, 1887-90. Zur Entwickelungsgesch. u. Anat. der ceylonischen Blindwühle, Ichthyophis glutinosus.

SELENKA, EMIL, 82.—Der embryonale Excretionsapparat des kiemenlosen Hylodes martinicensis. Sitzungsber. d. kön. preuss. Akad. d. Wiss. zu Berlin, 1882, p. 117.

STEENSTRUP, 46.—Rapport sur L'histoire naturelle de quelques Amphibes de Danemark. Kiel, 1846.

WILDER, H. H., 99.—Desmognathus fusca (Rafinesque) and Spelerpes bilineatus (Green). Am. Nat., XXXIII, p. 231.

WILSON, E. B., 94.—The Embryologial Criterion of Homology. Biol. Lectures delivered at the Marine Biol. Lab. of Wood's Holl, 1894.

ZIEGLER, H. E., 02.—Lehrbuch der vergl. Entwickelungsgesch. der niederen Wirbeltiere. Jena.

EXPLANATION OF PLATES.

The figures are taken from camera drawings. The embryos are magnified in Figs. 1 to 10, inclusive, about thirteen and a half diameters, in Figs. 11 to 14, inclusive, a little more than thirteen and a half diameters, and in Fig. 15 about twenty-eight diameters.

PLATE I.

FIG. 1. Two-celled stage. Upper pole showing the beginning of the second furrow.

FIG. 2. Two-celled stage. Lower pole.

FIG. 3. Later stage of segmentation. Upper pole.

FIG. 4. Same egg as in Fig. 3. Lower pole.

FIG. 5. Stage showing blastopore.

FIG. 6. Stage I. Young embryo with the blastopore almost closed.

FIG. 7. Stage II. Posterior end of the embryo, showing the anus.

FIG. 8. Stage IV. Head end.

FIG. 9. Same embryo as in Fig. 8. Tail end.

FIG. 10. Stage VI.

PLATE II.

FIG. 11. Stage IX, as seen from the side.

FIG. 12. Stage XII.

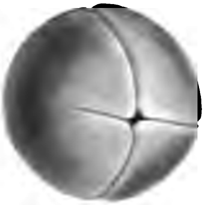
FIG. 13. Stage XIII.

FIG. 14. Stage XIV.

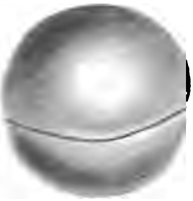
FIG. 15. Stage VIII. Anterior end of the embryo, as seen from the ventral side, when cut off the yolk. Y, point where the yolk has been cut away from the gut.

THE EMBRYOLOGY OF HYLODES MARTINICENSIS **PLATE I**

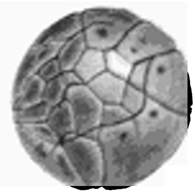
L. V. SAMPSON



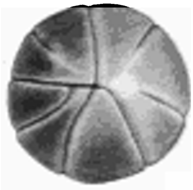
1



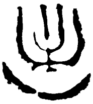
2



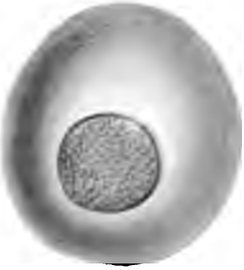
3



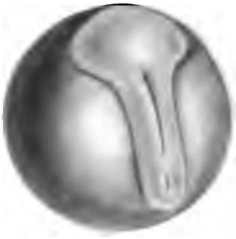
4



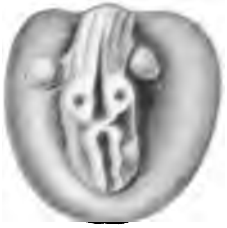
7



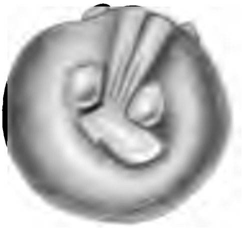
5



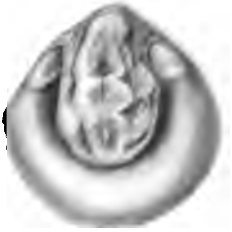
6



8



9



10

The Relation Between Normal and Abnormal Development of the Embryo of the Frog (III), as Determined by Some Abnormal Forms of Development.

By

T. H. Morgan.

With Plates XXXI and XXXII.

Eingegangen am 25. April 1904.

The following observations and experiments were made for the most part during the spring of 1903. Although the different points taken up under each heading are not closely connected, yet they all have a direct bearing on the general problem included in the title of the paper, which is the third of the series dealing with these questions. The eggs were in all cases those of *Rana palustris*.

Abnormal and Irregular Forms of Cleavage.

Several observers have already described cases in which the first cleavage of the frog's egg divides the egg into unequal parts. I wish to record here two extreme cases of this sort in which the relation of the first cleavage to the gray area was noted. The eggs were also isolated and found to give rise to normal embryos.

In the first case, Fig. 1, the first furrow extended from the top of the black hemisphere over towards the region of the gray area. The first two cells were, in consequence, very unequal in size; the smaller being almost entirely black, but containing at its lower end a little of the gray area. When next examined the egg was in the eight-cell stage, and had four small black cells and four large black and white cells, Fig. 2. There can be little doubt that the two cells, first formed, had divided twice each time into nearly equal parts. The dorsal lip of the blastopore appeared later on the gray side of the egg. It is clear, therefore, that the first furrow and the dorsal lip did not coincide in position, as is the rule for the normal egg, nevertheless a normal embryo developed.

Another egg, Fig. 3, did not divide quite so unequally. The second furrow came in at right angles to the first. The third furrows divided the small cells into unequal parts, Figs. 4, while the two large cells had begun to divide, when the observation was made, by nearly vertical furrows, Fig. 5. A normal embryo developed here also.

These two cases support the conclusion, first expressed by PFLÜGER, that the cleavage-form, as such, does not determine the position of the embryo on the egg. In the light of more recent results in experimental embryology it is becoming more evident that the distribution of the protoplasm is the factor that determines the position of the embryo on the egg and not the position of the planes of division. It is true that the cleavage planes often follow the lines of separation between the different protoplasmic areas, and hence appear at times to separate the egg into its constituent parts, but it has been found that the position of the cleavage planes can be shifted and the embryo may still follow the protoplasmic fields, rather than the position of the cleavage planes. It is, in fact, more probable that the position of the protoplasmic areas (by directing the spindle) influence the position of the planes of cleavage than that the planes of cleavage determine the location of the material of the embryo. This point will be further discussed below.

Two other unusual forms of cleavage were found in another bunch of eggs. The first of these is shown in Fig. 6, and in side view in Fig. 7. One cleavage furrow, 1-1, divides the egg into equal parts. This I shall arbitrarily call the first furrow. Another furrow, 2-2 (arbitrarily called the second here), starts above in the black hemisphere, but very much to one side. It does not extend down far into the egg. The two large black cell also show a plane of cleavage, 3 (called here the third furrow), which is nearly horizontal. It, also, does not extend far into the egg. Sections were made of this egg in order to see if the nuclei would give any clue as to what the peculiarities of the cleavage were due. A comet-like line of pigment extends from the cell-wall in the upper hemisphere into one of the blastomeres and probably represents the path of entrance of one spermatozoon at least. In each of the large blastomeres, and in the region of the third cleavage, an elongated pigment band indicates the division of a nucleus. In the small blastomeres on the crescent side of the egg there is a faint indication of a nucleus (?) in each. The most plausible explanation of this condition is, I think, that two spermatozoa have entered the egg and brought about its irregular division. In the other egg, Figs. 8 and 9, the first plane of cleavage, 1-1, is the only one that is completed. It divides the egg into nearly equal parts one of the halves containing all of the gray area. Each of the first two blastomeres appears to have two divisions in it, indicated by the letters 2-2, 2-2. Whether these appeared in succession, or whether, as seems to be the case, they appeared at the same time in each of the cells, can not now be determined. Sections of this egg show that nuclei (indicated by the balls of pigment) are present in all of the subdivisions of the egg. Near the top of the large blastomere there are two paths of pigment that appear to indicate that two spermatozoa have entered the egg. If this is the case the irregularities of the divisions are due here also to the presence of two spermatozoa in the egg.

These two cases, in which the abnormal cleavage is probably due to polyspermy, are entirely different, therefore from the first two cases described above, and also different from the cases that have been recently described in a paper by Boring and myself[1]. Since the general questions connected with the variations in the cleavage that we described were not there discussed, I propose to give a further consideration to some of the results here.

We found in about $8^1/_2$ per cent of cases, that the first cleavage plane came in at right angles to the median plane of the gray area, and therefore in the position of the second plane of the »normal« division. In eggs of this sort we found that the median plane of the embryo sometimes coincided with the first plane of cleavage, and sometimes with the plane of symmetry of the gray area, and in other eggs it lay between the two. The explanation of this difference is probably as follows: — It appears that the gray area is produced in the interval of time between the fertilization of the egg and the first division. It is probably produced as shown by Moszkowski by a rearrangement of the contents of the egg, — a streaming of the interior due to the action of gravity. When the first spindle is formed it becomes placed at right angles to what was the median plane of flow, so that the first plane of division will correspond approximately to the median plane of symmetry, i. e., it will divide the gray area symmetrically. The position that the spindle has assumed at right angles to the plane of symmetry of the protoplasm is, I assume, brought about by the elongation of the astrospheres at right angles to the plane of symmetry. The flow of the protoplasm has probably come to an end in the normal egg before the elongation of the spindle occurs, so that the spindle takes the position just described without being influenced directly by the movement. If, however, after the gray area has been formed, or while it is forming, the eggs should be turned (due to the bunch as a whole shifting its axis) the flowing of the protoplasm may begin again, and may so affect the nucleus that is assumes a new position before division. Hence the first plane of cleavage may appear in a different meridian of the egg.

---

[1] This Archiv. XVI. 1903. p. 682—690.

From the usual position of the spindle in the normal egg it appears that the spindle becomes placed in a position at right angles to the plane of symmetry of the protoplasm. The only assumption that we can offer to account for this is that some sort of regulation exists in the egg that causes the asters at the poles of the spindle to elongate across the plane of symmetry, and this movement of the asters will cause the spindle to lie also across the symmetrical plane of the egg. On the other hand we have assumed that this regulation may be easily disturbed if streams are set up in the protoplasm at the time of or after the elongation of the spindle. That this is not an arbitrary assumption is shown by the experiments of Pflüger and of Born on eggs turned into oblique or inverted positions. The flowing of the protoplasm that then occurs is known to carry the nucleus into a different part of the egg, and the position of the plane of division, that may come in while the protoplasm is still moving, shows that the position of the spindle is affected by the direction of the flow. It appears in some cases at least that this effect is such that the long axis of the spindle is in the direction of the axis of flow of the protoplasm. The spindle may be looked upon, under these conditions, as an elongated body floating in a more fluid substance. Such a body will tend to become placed with its long axis in the direction of the stream.

I believe we can profitably apply these suggestions to the type of eggs in which the first cleavage is at right angles to the plane of symmetry of the gray area. I interpret the position of the first plane of cleavage in these eggs as due to a secondary rotation of the protoplasm of the egg after the gray area has been formed. This rotation, as I have said, may suffice to carry the spindle into a new position; the rotation itself being due to a change in the position of the egg, which the egg has failed to meet by rotating on its axis, as usually takes place when the eggs are turned into a new position.

Now the median plane of the embryo appears to be determined by the symmetrical distribution of the protoplasm. In these eggs, in which the first plane of cleavage comes in at right angles to the normal position, there will tend to be two planes of symmetry in the protoplasm, namely, the one first formed by the gray area, and the new one due to the secondary rotation of the contents of the egg. Whichever of these two exerts the stronger influence will determine the median plane of symmetry of the embryo. In some cases it will be the first, in others the second, and in still others the median plane will come to lie between the two. Hence the median plane of these embryos should be expected to coincide sometimes with the median plane of the gray area, and sometimes with the first plane of cleavage (i. e., at right angles to the gray area), and at other times to lie between the two. This is what we actually found to occur. Thus the same hypothesis that explains the shifting of the spindle and the abnormal position of the first plane of cleavage will account for the change that sometimes, but not always, takes place in the position of the median plane of the body without supposing that the two are related as cause and effect. The connection lies deeper, namely, in the secondary change in position of the protoplasm that carries the spindle into a new position on the one hand, and produces a new plane of symmetry on the others, the latter change having a greater or less influence on the position of the median plane of the embryo.

It was also pointed out by Boring and myself that the third planes of cleavage are sometimes vertical instead of horizontal, and we found that this occurred most often on the side of the egg containing the gray area. In one bunch, in particular, a large number of such cases occurred. The explanation of these facts is probably as follows. The bunches of eggs were brought into the laboratory sometimes soon after they had been laid and before the jelly had swollen to any great extent. The bunches were often placed in shallow dishes. Under these circumstances compression of the eggs would almost certainly take place from above downwards, and this would affect the position of the cleavage planes, as Born and Hertwig have clearly shown. Furthermore the gray side of the egg is the side that is nearer the top of the egg than is any other part of the dark hemisphere, hence the vertical compression would be most likely to be shown on this side, and therefore the more frequent occurrence of the vertical cleavages in this region.

PFLÜGER in 1883 concluded that the spindle in the compressed egg would take the position of least resistance. HERTWIG later modified this »rule« so that it read; — the spindle assumes the position of the greatest protoplasmic mass. Numerous exceptions have been found to this statement. In the case of the sea-urchins egg I pointed out[1]) that this rule does not apply to the three-fold type of cleavage, and that in these the second planes of cleavage are clearly determined by a relation to preformed areas in the protoplasm. We are justified I believe in extending this conclusion to other, and perhaps to all cases, in the following sense. The position of the spindle itself is actually determined by movements of the protoplasm as admirably shown by CONKLIN's recent observations on the egg of *Crepidula*. These movements of the protoplasm are in turn determined probably by the condition of the protoplasm in each cell as a result of which at certain times currents are set up, and it is these that carry the spindle into position. How far the basis of this arrangement depends on the structure of the protoplasm, and how far purely on a difference in composition of different regions can not at present be determined. Both factors may enter into the problem. The differential divisions of the protoplasm that so often occur may be caused by the flow of the material, and as a consequence determine the position of the spindle. Thus there must be a finely adjusted set of conditions in the protoplasm that regulate the position of the spindle and the consequent position of the cleavage planes, but this regulation may be little more than the outcome of physical and chemical forces in the widest sense of the terms. Any attempt to refer the position of all spindles to a single factor as PFLÜGER and HERTWIG have attempted to do, is almost certain to fail, for, in different cells the conditions that determine the direction of the flow of the substances that give direction to the spindle may be of very different kinds.

---

[1]) This Archiv. 1895. II. p. 75.

Is Gravity Essential for the Formation of the Plane of Bilateral Symmetry in the Frog's Egg?

I do not propose to consider here that side of this question that has been discussed by ROUX and by SCHULTZE, but only the recent experimental evidence, in regard to the action of gravity, brought forward by KATHARINER ('02), MORGAN ('02) and MOSZKOWSKI ('02, '03). Furthermore I shall confine my discussion to certain questions raised by MOSZKOWSKI in his last paper, in which he brings forward certain evidence which he believes controverts the interpretation that KATHARINER and I have given to our results. It will not be difficult to show, I believe, that MOSZKOWSKI has failed to substantiate his claim.

The evidence that MOSZKOWSKI brings forward to show that the experiments of KATHARINER and myself are inconclusive is two-fold. In the first place he claims that in our experiment a centrifugal force has been substituted for that of gravity, and has been the factor that has determined the median plane of the embryo. Now this assumption implies that the eggs must have rotated for some little time in the same plane, and this may actually occur, MOSZKOWSKI claims, in bunches of frog's eggs rotating in a whirl of water. I do not question that this might happen, and presumeably did happen in MOSZKOWSKI's experiments. Whether it did, or did not, in KATHARINER's experiment, I will not pretent to say, but it seems to me improbable that so acute an observer could have overlooked so obvious an objection. In my own case I can state that I paid attention to this point and observed that the eggs did not rotate for any length of time in the same plane. The eggs of the toad are laid in strings, not in bunches as are the frog's eggs. These strings were cut up into short pieces of varying lengths. The rotation of these strings in the whirl of water was irregular in the extreme, and resembled a mass of living reptiles crawling in and out of the mass so that the individual eggs were carried through most complex and ever changing revolutions. I closely watched individual eggs and saw that they were constantly changing the plane of rotation as they slowly revolved in the water.

In the second place MOSZKOWSKI states that by turning on the water so that a strong current was produced he got abnormal development (protruding yolk for instance), which he says was due to centrifugalizing the eggs. Now this is a question with which I am especially familiar. Until Moszkowski states how many revolutions his eggs made in a minute, and their distance from the center of revolution, it will not be possible to determine whether or not his results are in reality due to a centrifugal force. I may recall that I have found that the eggs of *Rana palustris* may be rotated at the rate of 140 revolutions per minute at a distance of from 7$^1/_2$ to 19$^1/_2$ inches from the axis of rotation without causing them to develop at all abnormally. It would be interesting to know if Moszkowski rotated his eggs as rapidly as this, and if he did, the rate is so enormously greater than that which I used for the toad's eggs that it is quite inconceivable that the two results can in any way be compared. It is no less important to remember that this form of abnormality, which Moszkowski says is due to centrifugalizing the eggs, occurs whenever the yolk cells are injured in any way as I have shown a number of different times. Rough handling, or perhaps even irregular shaking, if sufficiently violent, might produce exactly the same result as a strong centrifugal force. Therefore while it is perfectly possible that Moszkowski's abnormal embryos may have been due to the centrifugal force, the fact that this is the case is not sufficiently shown by the evidence that he has given.

To return to my own experiments with the toad's egg. That the results here were not due to the centrifugal force is shown I believe first by the irregularity of the rotation (as stated above), second by the entire absence of the gray area, or of a similar appearance on the eggs as they developed. Moszkowski overlooks the fact that I took a few eggs out at intervals and examined them for this very point. Third that the development was normal in all respects and showed in no way that the eggs had been centrifugalized. The first and the second point suffice, however, to establish the conclusion that I drew from the experiments.

Finally it seems to me that while Moszkowski has brought forward strong evidence to show that gravity is the most important factor under normal conditions in determining the arrangement of the protoplasm and in locating, in consequence, the median plane of the embryo, — (a further analysis of this relation I have attempted to give in the preceeding section for certain abnormal cases), — yet it appears to me that he has arbitrarily assumed that gravity is the only factor that can produce this result in the frog's egg. In other eggs we have strong evidence to show that gravity plays no part in the determination of the median plane of the embryo, and in these cases other factors must assume this function.

Now while I am willing to admit that gravity may play an important role in the frog's egg, it does not follow that, when the action of gravity is excluded, the egg may not have other means of determining the middle plane of the embryo. In fact, we do not have to look far to find other factors that might produce this result. The entrance of the spermatozoon may, as claimed by ROUX, be a factor, if in no other way than as the outcome of the apposition of the two pronuclei and the position assumed by the asters in the plane of apposition, which will give a plane of bilateral symmetry that coincides in position with the point of entrance of the spermatozoon. Whether or not ROUX's experiment of localized fertilization really establishes the conclusion that he has drawn from it, is a point that future experiments must decide.

The egg is at first attached to the wall of the ovary by one side, and it is not impossible that the point of attachment may, through a possible influence on the distribution of materials, produce a bilateral condition, or at least be an influence that may become a factor, if stronger influences are eliminated.

For these, and for other reasons that I need not go into here, I still believe that the evidence in favour of the view that the toad's egg, and probably also the frog's egg, can develop normally when the action of gravity in one plane is removed (and is not replaced by a centrifugal force) is valid.

The Effect of Killing One of the First Two Blastomeres Before the Cleavage is Completed.

ROUX's experiment of sticking one of the first two blastomeres has lead to so much controversy that it may not seem surperfluous to describe an experiment in which a variation of this method led to an unusually definite result.

The question suggested itself: — what will occur if an egg is stuck at the time when the first furrow has just appeared? Will the division be stopped? If so and if at the same time one of the first two nuclei should be killed, or injured, there might result a whole egg containing the nucleus of only one of the first two blastomeres. Under such circumstances the development of the egg might give some points of interest. As a matter of fact it was found, as has been more fully described in my paper with Miss Torelle, that the first cleavage generally completes itself, so that the main purpose of the experiment can not be attained in this way. Indirectly, somewhat the same result is at times obtained by the breaking down of the cell-wall between the first two cells. If, however, the nucleus of the injured cell is not killed, as is nearly always the case, it may continue to divide and supply the injured half of the egg with the nuclei around which cell-walls may subsequently be formed, and this half become added to that of the uninjured half. In one egg, stuck at the time of appearence of the first furrow, the injury was so severe that the nucleus was seriously injured and failed to supply the injured half with nuclei. It divided very irregularly, and only a very few times, and the resulting nuclei remained near the place of injury. They did not become the centers of cell-formation. It was also evident that the part that developed was less in volume than half of the egg, which was due, most probably, to the cell-wall between the blastomeres breaking down so that the protoplasm of the lower part of the blastomere became continuous with that of the injured side, and failed to become supplied with nuclei in the later stages of development. Possibly in this case the cell-wall between the first two blastomeres had not completed itself which would lead to practically the same conditions in the egg as though it had first formed and then broken down.

A surface view of the egg, at the time when the embryo appeared, is shown in Fig. 16. A small »half-embryo« is present on the uninjured side along the line of union with the injured half. The embryo appears to be somewhat less in size than a half-embryo, and this is due, no doubt, to loss of material from the uninjured side. The half medullary plate that is seen in the figure is somewhat broader at its anterior end where it is arched over to some extent by the yolk of the injured side. It is a point of interest to note that the medullary plate lies on that side of the egg that is opposite to the upper injured hemisphere of the other blastomere, which means that the embryo has formed over the yolk hemisphere of the egg. The egg was cut into cross-sections, three of which are shown in Figs. *G*, *H*, *I*. The first of these is through the anterior end of the medullary plate. This appears (above in the figure) as a nearly bilaterally symmetrical structure, lacking a little on the inner side. In sections further forward, however, the medullary plate becomes quite bilaterally symmetrical. Beneath the medullary plate lies the mesoderm, extending as a thick sheet over one side while on the other, although it extends over the middle line, it is much less developed and does not continue downwards along the inner side at all. In the middle of the yolk the small opening of the archenteron is present. The outer surface of the embryo is covered by ectoderm, which in this section extends on the inner side between the embryo and the injured half.

The injured part of the egg, as shown in this same section, contains a few irregular and abnormal nuclei especially in the region near the point of injury. The protoplasm is much vacuolated and may be dead in some places.

Passing along the series of sections towards the middle of the embryo, the ectoderm over the inner side is found to become thinner, and finally to come to an end over this side, as in Fig. *H*. The archenteron extends towards the inner side and opens out as shown in this figure. It is important to note that its roof is bounded by yolk-cells (endoderm), and that these are continuous near the opening with smaller cells, which are exactly like the mesodermal cells that lie on their inner side. Further out, along the upper, inner wall, the small cells grade off into the ectoderm, which still further is thickened to form the half-medullary fold. The notochord lies on the left side (in the figure) of the medullary plate, and is nearly surrounded by mesoderm from which it appears to have been cut off. It lies in the region where the ectoderm and the mesoderm of the of the surface become continuous. The mesoderm extends over the entire right side of the section, and is differentiating below from the yolk-cells.

Passing to sections further posteriorly we find that the archenteron extends much farther into the yolk, even more extensively in some of the sections than shown in Fig. *I*. The ectoderm of the medullary plate extends further over on the inner side, and finally (posterior to Fig. *I*) the medullary plate is nearly bilaterally symmetrical again. In these posterior sections the notochord, as such, can not be found, because, no doubt, it becomes merged into the mesoderm in this region of the embryo. The surface ectoderm has apparently met with an obstacle on the lower side and its cells are heaped up there. The yolk cells on the inner side are looser than in the more anterior regions, and, here and there, seem to be imbedded in the yolk that is continuous with that of the injured part; but the cells are always near, or are directly continuous with, the yolk-cells of the embryo, and there can be no doubt that their nuclei have originated in the uninjured side.

This series of sections is instructive in many ways, and I wish to point out here, as briefly as possible, some of the more interesting questions that are involved, in the formation of an embryo of this kind.

If an egg in which one of the first two blastomeres has been stuck is kept under observation at the time when the dorsal lip of the blastopore first appears, (and the same is true for an egg stuck at the time when the first furrow has just come in), it will be found that the dorsal lip develops as a small crescent on the side of the egg near the line between the injured and the uninjured halves. It then begins to extend around one side of the egg to form the lateral lip as shown in Figs. 13, 14, 15, and at the same time the dorsal lip advances backwards over the surface of the yolk. The lateral lip has begun meanwhile to roll over the yolk also, and the ventral lip, that has now appeared, extends forwards for a short distance. In other words the uninjured half follows the same method of development as half of the normal egg would do. The open side of the archenteron represents the edge of the lateral lip of the blastopore.

The anterior end of the medullary plate of the frog-embryo is laid down in the undivided material in front of the first position of the dorsal lip of the blastopore, and hence on that part of the egg not involved in the blastoporic overgrowth. It has been noted that the medullary plate in this half-embryo, especially at its anterior end, is a whole structure of reduced size, and not a half. This peculiarity of these half-embryos has also been noticed by Roux, but I think its importance has not been fully appreciated, for there is no evidence that it is due to an early postgeneration of the missing (?) half. As far as I have been able to determine, the anterior end of the medullary plate is laid down at once as a whole structure, and not as a half that completes itself on one side; hence I can see no advantage in calling the process one of postgeneration, since the half is no more postgenerated than is the whole embryo that develops from the isolated blastomere of the sea-urchin's egg. In both cases, no doubt, some change takes place, so that the middle line becomes formed some distance from the inner edge, but this involves, I think, a different conception of the process from that which supposes the half of the whole structure is first laid down, and new cells are then thrown across, as it were, to the other side to duplicate the half-structure that was first formed. It is this latter process, to which ROUX applies the term postgeneration. My own idea of what takes place involves the assumption of axial readjustment of material that is so little predisposed in one direction (half-structure) that its relations may be readjusted in response to the form of the developing part.

In the middle regions of this embryo the medullary plate is a half-structure, or at most a little more than half. The notochord, although rounded and appearing therefore as a smaller, whole structure, lies on the inner side of the medullary plate, and is, therefore, in the position of a half-structure. It lies just beneath the surface, and is, in part, surrounded by mesoderm. The surface cells over the notochord show a gradation between the ecto- and mes-endoderm. These cells, as well as those that lie further along over the inner surface of this region, call for special notice. It is this part of the surface that is rolled over in the normal embryo to form the roof of the archenteron. The cells in this region are those just outside of the lateral lips of the blastopore, and normally are brought together to unite in the advancing dorsal lip of the blastopore. These are superficial cells that lie at first outside of the rim of the blastopore, but whether, therefore, we shall call them ectoderm or endoderm is, I think, entirely a matter of definition. If we determine to call them ectoderm, then we must conclude that part of the cells of the dorsal roof of the archenteron are at first ectodermal. It has been shown by SCHWINK and by KING that some of the cells of the roof of the archenteron are added to the notochord and to the mesoderm. Whether the dorsal wall, after this process has taken place, becomes purely endodermal, or whether some of the ectodermal cells may remain, can not be definitely stated. The distinction is perhaps largely artificial, for, in the lips of the blastopore, the ecto-, meso-, and endoderm are so closely united that it is probable that all the cells of this part have the potentialities at first of all three layers. Thus the notochord may be ascribed to the ectoderm, or to the mesoderm, or to the endoderm, at will, and it may be a matter of indifference to which layer we ascribe it, since the cells of these layers in the part of the embryo from which the notochord develops may all have the potentiality of producing this structure should they happen to get into the part from which the notochord is formed.

There is another point shown by this half-embryo that calls for a brief mention. The roof and the floor of the half-archenteron are formed from the yolk-cells, and the archenteron is produced here not by the rolling in of the edge, although this also takes place, but by the yolk-cells withdrawing and opening out. This part of the archenteron corresponds to the lateral portion of the archenteron of the normal embryo, which, I believe, is also formed by the withdrawal, or pulling in of the yolk-cells along the lateral lips of the blastopore. It is also probable that the same process occurs at the dorsal lip of the blastopore, as claimed by ASSHETON (though not so extensively, I believe, as he supposes). It is, of course, hazardous to argue from the conditions in the half-embryo to the normal development, but in the two cases the processes have every appearance of being the same, and in the half-embryo the conditions are such that there can be only one interpretation as to how the result has been attained; hence it appears to me not unreasonable to extend cautiously the same interpretation to the normal embryo where the result is more obscured by other conditions.

Finally I would recall that the half-embryo in this case is less than half in volume, due apparently to the loss of yolk material from the uninjured blastomere. This seems to have occured in the yolk-region of the more posterior part or the embryo, so that the equatorial zone from which the embryonic material is mainly derived has lost relatively less. The extent to which the yolk becomes nucleated from the nuclei derived from the uninjured half will determine how much of it becomes cellulated. In this particular case the nuclei do not appear to have extended over into the injured portion, although in consequence of the continuity of the yolk in this region the nucleazation might, as far as we can see, have been more extensive. As soon however as the protoplasm becomes broken up into cells around these nuclei, further nucleazation will come to an end, and this is what actually seems to take place in eggs of this sort. From this point of view the in-wandering of nuclei from the uninjured into the injured half, as ROUX claims to take place, is not likely to occur after the protoplasm has once become broken up into cells.

There is a question of theoretical interest in connection with the postgeneration of the injured blastomere which brings to light a difficulty of fundamental importance. If the »half-embryo« lies in large part over the lower hemisphere, as ROUX believes, then there is opposed to the »open« side of the half-embryo the large yolk-cells of the lower hemisphere, as shown in Figs. 13, 14, 15. How is it possible that this material can be transformed into the dorsal side of the postgenerated »half«, even under an influence emanating from the developed half, as Roux supposes. It must be remembered that the material to form the embryo proper is carried downwards by the overgrowth of the dorsal and lateral lips of the blastopore and has an origin relatively high up on the egg. The yolk-cells of the lower hemisphere form the floor of the archenteron, and are removed by half the diameter of the egg from those yolk-cells that form the mesoderm of the embryo. Hence it seems to me, in the light of recent advances, highly improbable that Roux's account of this process can be correct.

Closely connected with this question there is another, which, I believe, has not been sufficiently considered. According to Roux the characteristic process of blastoporic overgrowth, by means of which the material of the embryo is carried over the lower pole, is ommitted in the postgeneration of the injured half. For several reasons this does not appear to me to be possible. If, on the contrary, we suppose that the injury has only sufficed to delay the development of the injured half, is it possible that the uninjured half may first complete its gastrulation, and then the delayed half pass through the same process? There is no evidence that this occurs. If it did so, it would, no doubt, have been observed. Is it possible then that the development of the uninjured half is held back until the process of gastrulation can go on simultaneously in both halves? This may sometimes occur, but gives rise to spina bifida embryos, or even to normal embryos if the yolk has not been much injured. It does not appear that the »postgeneration« that Roux describes takes place by either of these two processes. There remains another possibility. The material for the middle and for the posterior regions of the body may be supposed to grow backwards from the head region of the half-embryo, i. e., the material of the injured half that lies at the side of the half (?)-head may be imagined to extend backwards in somewhat the same way as the dorsal lip extends backwards, without, however, an actual process of gastrulation taking place. It seems to me that we also lack evidence to establish this view. From the proceeding analysis it appears improbable that the material of the injured half can be used to replace the missing half-embryo unless it passes through a regular process of gastrulation. The half-embryo can at most only partially complete itself by a sort of lateral regeneration (involving to some extent the process of morphallaxis) of its own tissues. This view seems to me the most plausible one in the light of the experiments that have been so far carried out.

The Size of the Cells of the Embryo, and the Size of the Egg.

The eggs laid by each individual frog are usually all of about the same size within rather close limits. This size is often variable for different individuals of the same species. Every embryologist must, however, have found occasionally bunches of eggs in which smaller eggs than the rest of the batch are present. There is an interesting theoretical question connected with these small eggs, to which I have already called attention[1]. I described some cases, in which small eggs of the toad were found that contained only one-half of the volume of the normal eggs, and which, therefore, were equivalent in volume to one of the first two blastomeres. During last spring I found in one bunch of frog's eggs a few eggs that were so small that they at once arrested my attention. Measurements of these eggs showed that some of them contained no more than half of the volume of the other eggs of the same bunch. The following figures give the measurements of some of the small, dwarf eggs, and, for comparision, the measurements of some of the normal eggs.

Normal Eggs	Dwarf Eggs	
50 × 53	37 × 39	42 × 45
52 × 53	40 × 42	43 × 43
50 × 52	42 × 42	44 × 45
49 × 52	44 × 44	42 × 44
49 × 51	44 × 47	42 × 43
50 × 53	40 × 42	45 × 46
50 × 50	44 × 46	

If the cubes of the diameters of the dwarf eggs be compared with those of the full-sized eggs, the volumes will be found to be as 1 to 2. The small eggs were isolated and produced normal embryos, also of reduced size. These were preserved at different stages along with the normal eggs, and, later, sections were made of both kinds, always carrying one of the small eggs and one of the large eggs through the same solutions, etc., and using only these for comparison. Camera drawings were then made of cells from corresponding regions of the two embryos of each pair, and the drawings compared.

---

[1] This Archiv. 1901. p. 429.

Before speaking of the results there are a few points in regard to the origin of these dwarf eggs and their mode of cleavage that must be described. If the ovary of a frog be examined during the winter the full-sized eggs are found hanging from the walls of the ovary into the central cavity. In addition to these there are other eggs of all sizes, especially very small ones, that will become the ripe eggs of the following year. The dwarf eggs appear to have been some of the eggs that had not quite reached maturity (not the very smallest just spoken of). The pigment in these immature eggs is not so deep, nor so extensively developed, as it is in the large eggs. One of the most evident characteristics of the dwarf eggs also is their light color. It seems to me, therefore, probable that these eggs correspond to the small eggs of the ovary that have been accidentally set free, possibly because they were large enough to »ripen« at the time when this process occurred in the ordinary eggs.

In several cases I followed the process of cleavage of the dwarf eggs. It seemed at first sight to depart widely from the normal type of division. The first division separated the black part of the egg from the white, and appeared to lie therefore in the position of the third plane of cleavage of the normal egg. As shown in Fig. 10 the first plane of cleavage extended between the white and black parts of the egg, beginning at the upper edge of the black. Possibly these eggs were not free to rotate in their membranes, and consequently a rearrangement of the contents of the egg took place beneath the surface. This view is supported by the fact that the following sequence of the divisions is as in the normal egg. The second furrows came in at right angles to the first, Fig. 11. The third furrows, in the few cases that were observed, cut off four upper smaller cells, two of which were dark and two light, Fig. 12. Thus the divisions are, as I have said, the same as in the normal egg, although the distribution of black and white is different. The results resemble those that Pflüger obtained when he forcibly held eggs in oblique positions. The medullary plate when it appeared was markedly lighter than in the normal egg, which may be due, in part, to the lack of pigment in the egg itself, and also, as in Pflüger's eggs, to the rotation of the interior.

Some of the embryos were killed at the time when the dorsal lip of the blastopore first appeared; others at the time when the medullary folds were just outlined on the surface of the egg, and others when the medullary folds had closed and the tail-knobs were just beginning to form.

It was by no means an easy matter to decide in all cases whether the small eggs produced the full (normal) number of cells, which would therefore be smaller, or only half as many cells of the same size as the normal. The difficulty arises mainly from the irregular form of the cells of the embryo and from the uncertainty in locating exactly similar regions of the two embryos. For these reasons the following results are not as final as I had hoped that they might be.

It was found that the cells that make up the roof of the segmentation cavity, at the time when the dorsal lip of the blastopore has just appeared, are smaller in the dwarf embryo than in the full-sized embryo. There is also a considerable and well-marked difference in the size of the yolk-cells. The roof of the segmentation cavity is also narrower in the small embryos. In some of the pairs the difference in the size of the cells of the roof of the segmentation cavity is so slight that it does not appear to be as great as was the original difference in size between the eggs themselves, but this is a point most difficult to determine.

At the time when the blastopore finally closes the ectoderm cells of the medullary plate and the underlying mesoderm and endoderm cells are smaller in the dwarf embryos and the yolk-cells of the yolk-mass are very decidedly smaller.

In older embryos I have found it difficult, owing to the small size of the cells, to make out any decided difference, but I have no reason to suppose that the conditions here are different from the preceeding, only that as the cells get smaller the difference in size is harder to detect.

From these observations, that need not be given in extenso, it appears that the smaller eggs produce smaller cells. The evidence is not sufficient to establish, however plausible it may appear, that the cells are proportionately smaller. In fact, the evidence, as far as it goes, rather indicates that the cells stand somewhere between the normal and the proportionately reduced size.

The same theoretical question that is here involved I have already considered from two other points of view. I compared the size of the cells of a half-embryo of the sea-urchin, that had developed from one of the first two blastomeres, with the size of the cells of the normal embryo, and found that the half-embryo is made up of cells of the same size as those in the normal, whole embryo. There are, therefore, only half as many cells in the half-embryo and these are, in comparison with the normal embryo, twice too large.

In the second place I discovered that there is a definite relation between the number of times that the segmentation nucleus divides and the size of the piece of protoplasm in which it is contained. The eggs were shaken into pieces, after they had been fertilized, and the development of pieces of different sizes was followed. It was found that the number of times that the segmentation nucleus (and its products) divide is strictly determined by the size of the piece — the larger the piece the greater the number of times it divides. I also observed that even at the two-cell stage of a fertilized fragment the new nucleus in each blastomere is smaller than the nucleus of one of the first two blastomeres of the normal egg. In other words, the size of the nucleus is influenced by the size of the cell in which it is contained, even when the number of chromosomes remains the same. This relation has often been observed and commented upon by workers in the field of cell-lineage, but the experimental proof shows that this relation is not a regulation that is peculiar to the kind of division that is taking place, was might be claimed for the cases of normal cleavage, but is due directly to a regulation between the amount of protoplasm and its contained nucleus.

Comparing these results from the sea-urchin's egg with those described here for the dwarf eggs of the frog we find the following differences in the two cases. The isolated blastomere of the sea-urchin divided as many times as it would have done had it remained in contact with its fellows. The number of its divisions appears to be regulated by a size-relation between the nucleus and the protoplasm around it. Both the nuclei and the cells become smaller as the cleavage proceeds, but the cells are reduced in size proportionately faster than the nuclei. At last a stage is reached when a certain fixed relation of size is attained when further division for this stage of the development comes slowly to an end. The nucleated fragment of an egg also continues to divide until the same relation is established (without having divided as many times as the whole egg divides). The cells that result are, in relation to the size of the piece, proportionately larger than those of the whole egg (although they may be actually even a little smaller). This may be connected with the reduction in size of the first formed nuclei after the first division. All the subsequent nuclei are smaller than those of the same divisions of the full-sized egg.

The dwarf eggs of the frog, on the other hand, produce cells smaller than the normal; i. e., they appear to be organized on a smaller scale, and tend to produce approximately the same number of cells that are present in the full-sized embryos. Whether they actually do produce as many cells, or only show a tendency in that direction, is, as I have said, difficult to decide with certainty. The outcome, however, appears to be in principle different from that in which a part (isolated blastomere or egg-fragment) develops. In the latter case the cells are of the same size as are those of the whole egg, and are therefore proportionally larger for the size of the small embryo than are the cells in the whole embryo. In the small, dwarf egg the cells tend to become proportionally of the same size as are those of the full-sized embryo.

Extreme Forms of Spina Bifida (Ring-Embryos).

One of the most common forms of abnormal development of the frog's egg is brought about by the failure of the lips of the blastopore to unite over the yolk, which is the result of the inability of the yolk to draw inwards at the time when the blastoporic lips are ready to move forward. All stages in the extent to which this process occurs may be found in almost any series of eggs in which the yolk has become injured by one means or by another. The most extreme cases are those in which the halves of the embryo are completely separated, uniting only at the head and tail ends as described by Roux ('88), Hertwig ('92), and myself ('93). From the evidence furnished by these embryos Roux concluded that the primordium of the embryo lies around the equator of the egg, and that the material out of which the embryo is formed extends at first on each side over nearly 180 degrees of the surface of the egg. To this conclusion I have taken exception. In the first place on the grounds that the embryo itself is not more than 120 degrees in length at the time when it first appears. In the second place an important consideration in the early development of the embryo has been overlooked. During the segmentation of the egg there is a transfer downwards of material around the sides of the upper hemisphere which is due, in part at least, to the enlargement of the segmentation cavity. There is also an extensive differentiation at the sides leading to the formation of ectoderm and of mesoderm below the equator of the egg. If either the downward transfer of material, or the later differentiation downwards of the ectoderm and mesoderm is prevented or delayed the embryo may appear higher up on the egg, — developing presumably, in part at least, out of the same material from which the normal embryo develops. Hence the position of these ring-embryos on the egg is not a safe criterion as to the position of the material of the normal embryo before the time that the medullary plate develops. If, however, we assume that the transportation of material downwards during the cleavage, and also the formation of embryonic material below the equator, is interferred with, we can explain how it is that the ring-embryos may actually appear above the equator of the egg.

Another question of interest in this connection is whether the material of the sides, when it is as widely separated as in these ring-embryos, may not form more than half an embryo on each side. I have shown, in fact, that when one side of the egg is seriously injured the other side may produce more than a half structure. In looking over a number of egg that had been rotated in a centrifugal machine (at the rate of 170 to 200 revolutions per minute, and at a distance from the axis of rotation of from 5 to 20 inches) I found the two embryos drawn in Figs. 18—20, 19—21, in which in surface view, as seen in these figures, it appears as though possibly more than a half of an embryo lies along each side of the exposed yolk. These embryos were examined by means of sections, and although it turned out that each side is not a whole structure, as the surface views might seem to indicate, yet some other peculiarities were observed that make it worth while, I think, to give a somewhat detailed description of the two cases. I have already described a number of embryos from the centrifugal machine, none of which were as extreme forms of spina bifida embryos as these. On the other hand I have obtained as extreme forms as these in salt-solutions, and even in bunches of eggs insufficiently supplied with oxygen.

The more extreme of these two embryos is shown in Figs. 17, 18, 20. The yolk portion of the eggs, when turned upwards as in Fig. 18, is surrounded by a thickened black border. This is the medullary plate with a groove along its upper surface. It is owing to this groove that each side has the appearance, in surface view, of forming a small, but whole medullary plate. There is another deeper groove, just within the border of the yolk, which sections show is the beginning of the archenteron of the sides. At the posterior end of the embryo the grooves of the sides come together, and a rather wide and deep pit is formed in this region.

This embryo was cut into cross-sections. Sections taken at any point in the middle region of the embryo are like the one drawn in Fig. *A*. In this it is seen that the yolk is exposed over a broad area between the two halves of the half medullary plates. The outer edge of the yolk just inside of the medullary plates dips downwards to form a rather deep groove, the archenteron. Outside of the groove there are a few meso-endoderm cells which are continuous with the ectoderm of the medullary plate. No notochord could be found in any of the sections. The mesoderm is a broad band lying between the yolk and the ectoderm.

Towards the posterior end the yolk-mass sinks down in the middle, the archenteric grooves at the sides become deeper; and still further back they extend across the middle line, as the yolk mass sinks down, and form here the deep groove seen on the surface. The two half, medullary plates meet behind this region. It is evident in this embryo that the halves are separated throughout their whole length, and that a whole medullary plate is not formed on each side as the surface view seemed to show.

It would be erroneous to conclude from embryos of this sort, as I have pointed out above, that in the normal embryo the medullary plate is formed out of material that is separated throughout its entire length, or that the halves of the embryo encircle as great an area of the surface of the egg as in this ring-embryo. Owing to the change in shape of this embryo its medullary folds appear longer than when first laid down. It is more difficult to account for the shortening of the anterior part of the medullary plate in this embryo (and this is a characteristic shown to a greater or less degree by all ring-embryos). The peculiarity is due, I think, most probably to the fact that the dorsal lip of the blastopore appears higher up on the egg than in the normal embryo, and this, in turn, is the result of the lack of downgrowth of material, as already described. In consequence much of the region out of which the head of the normal embryo is formed is not present in front of the dorsal lip of the blastopore, and as a result the anterior end is correspondingly shortened, although the sides of the embryo may be even longer than the normal. In a sense the material that forms part of the sides may represent, to some extent, material that in the normal embryo would become part of the head region, but only to a limited extent.

The other embryo has a smaller exposure of yolk, which is due largely to the advance of the ventral lip. In other respects the surface view resembles that of the last embryo. The cross-sections present several points of interest. A section through the anterior end of the medullary plate, where the sides have just come together, is represented in Fig. *B*. The medullary plate bends in here, and on one side the optic vesicle pushes out towards the surface. On the other side of this section there is a hollow ingrowth of the surface ectoderm that represents the blastema of the right ear, and a few sections further back the blastema of the left ear is present (the sections being cut somewhat obliquely). In sections further forward I can not identify any thickenings to form the lenses of the eyes. The infundibular ingrowth of ectoderm is well developed in these anterior sections. A few sections further back than the one here figured show the halves of the medullary plate drawing apart, and the notochord appearing on each side, as in Fig. *C*. Towards the posterior end of the embryo, Fig. *F*, the lateral grooves of the archenteron become deeper, the yolk projects less and finally the sides of the embryo come together across the middle line. The half medullary plates, that have become smaller, meet, and posterior to this region the deep groove seen on the surface is indicated by a depression of the ectoderm, Fig. *D*. In this region the mesoderm from the sides also meets across, and there is present a small archenteron in the yolk that opens out more anteriorly on the surface. A few sections further back, the surface groove deepens and then unites with the posterior extension of the archenteron, as shown in Fig. *E*. This opening is the anus. What appears to have happened in this posterior region is this: — A posterior backgrowth of the archenteron under the ventral lip has taken place. The sides of the ventral lip have grown forward and have met above the yolk, the groove on the surface indicating their line of union. Whether a posterior opening has remained behind, or a new one has formed cannot be determined at so late a stage. The groove on the surface can not be interpreted as equivalent to the very small groove in the normal embryo that appears behind the blastopore, after the blastopore, which remains for a time as the neurenteric canal, has become reduced to a small opening. The anus is formed, it is true, at the posterior end of the groove in the normal embryo as a new opening, but there was left no groove on the surface as the ventral lip advanced. In the abnormal embryo the large depression on the surface represents the line along which a fusion of the posterior sides of the embryo have united over the surface of the yolk and not the groove of the normal embryo behind the neurenteric canal.

ZIEGLER in his recent, »Lehrbuch der vergleichenden Entwickelungsgeschichte der niederen Wirbelthiere« describes the normal process of closure of the blastopore of the frog as taking place in the following way: — When the last trace of the yolk-plug has disappeared within the small blastopore the lateral lips of the blastopore come together and fuse, leaving the anterior end open as the neurenteric canal and the posterior end as the anus. The dark groove seen on the surface represents the line of union of the lateral lips. This description is erroneous for the frog, although it is exactly what takes place, as I have shown, for *Amblystoma*. I have examined a great number of sections through frog embryos at the time when the blastopore is overarched by the medullary folds, and find that when the neurenteric canal is just overarched (it is the only part of the blastopore that is left open after the yolk-plug has been withdrawn) and the anus has not broken through, the archenteron has no posterior opening. This could not be the case if ZIEGLER's description is correct. Moreover an examination of the relation of the anus to the archenteron will show that ZIEGLER's description must be wrong, for the anus opens at the posterior end of a posterior sac-like extension of the archenteron which lies some distance behind the first position of the blastopore. In 1889 I described this mode of development, and since then I have re-examined the same preparations and new ones also, and find my account entirely correct. SCHANZ had in 1887 given an exactly similar description of the closure of the blastopore which account by an unfortunate oversight I failed to see, and did not refer to in my paper. My results have, therefore, at least the value of independent observations, although the credit for first giving an exact account of what takes place belongs to SCHANZ. The ventral lip moves forward over the region in which the anus will form, and, in fact, helps to form the posterior pocket of the archenteron, (which is probably deepened also by the pulling in of the yolk-cells in this region). The anus is a new invagination, and the little groove on the surface is also new, although I will not deny that the groove may sometimes be faintly marked as the blastopore undergoes the final stages of its closure.

To return to the abnormal embryo represented in Figs. 18 and 19. I have pointed out that the anterior single portion of the medullary plate is very short, and yet the optic vesicles are present at its anterior end, and the infundibulum turns in just in front of the termination of the plate. It is evident, therefore, that this part must really represent the anterior end of the fore-brain. It will also be noticed that the two ears lie almost at the level of the optic vesicles, and are therefore very far forward as compared with the optic vesicles. This supports strongly the point that I have tried to make above, namely, that the brain region is very much shortened in these embryos. I have further interpreted this to mean that there has not taken place the normal downgrowth of material from which the anterior part of the medullary plate develops in the normal embryo.

Summary.

1) The eggs of the frog in which the first furrow divides the material as unequally as in Figs. 1 and 3 may give rise to a normal embryo.

Irregular division like that shown in Figs. 6 and 7 and in Figs. 8 and 9 is probably due to polyspermy.

An analysis of the results published by MORGAN and BORING[1]) suggests the hypothesis that those exceptional cases ($8^1/_2$ per cent) in which the first plane of cleavage is at right angles to the median plane of the gray area, are due to a secondary rotation of the contents of the egg that carries the spindle into a new position. The median plane of the embryo of the frog appears to be established by the plane of symmetry in the protoplasm, and may coincide in these cases either with the first plane of cleavage or with the median plane of the gray crescent according to which influence predominates; i. e., according to which plane of symmetry is the more potent.

The position of the spindle in the cell appears to be determined by movements of the protoplasm, and these in turn may be the result of different internal and external conditions. It is improbable that the cause of the protoplasmic movements is the same for all kinds of cells.

2) The evidence in favour of the view that gravity determines the position of the gray area, and hence of the median plane of the

---

[1]) This Archiv. XVI. 1903.

embryo of the frog, does not preclude the possibility that if gravity is removed, as in Kathariner's ('03) and in my own experiments ('03), the embryo may have its median plane determined by some other factors.

3) The half embryo represented in Fig. 26 was obtained by sticking with a hot needle one of the first two blastomeres before the first furrow had completed itself. The method of formation of an embryo of this kind, (see cross-sections *G*, *H*, *I*), is illustrated by the diagrams shown in Figs. 13, 14, 15. The head of the embryo is nearly a whole structure and this is not due to a half head being first laid down which then completes itself on the inner side, but the head is from the beginning laid down nearly symmetrically in the material in front of the first position of the dorsal lip of the blastopore.

An analysis of the conditions present when a half-embryo develops leads to the view that when such a half-embryo is first formed the uninjured material of the other side can not be used to produce the missing half, unless it has also first gone through the regular process of gastrulation at the time when the uninjured half has gastrulated.

4) Dwarf eggs of the frog are sometimes found whose volume may be no greater than half that of the normal egg. These dwarf eggs correspond, therefore, in volume to one of the first two blastomeres. They give rise to normal dwarf-embryos. The cells in these dwarf-embryos are smaller than those in full-sized embryos, indicating that the small eggs tend to pass through the same number of divisions as does the normal egg. The isolated blastomere of the sea-urchin's eggs on the contrary produces only half as many cells as does the whole egg.

5) Extreme forms of spina bifida, — ring-embryos —, show that the medullary plate may be divided to nearly its anterior end. It would be erroneous to conclude from this condition that in the normal embryo the dorsal lip of the blastopore begins near the anterior end of the brain and that the rest of the embryo is formed by concrescence. The difference in the two cases can be accounted for on the assumption that in the ring-embryos the downgrowth of material during the late cleavage-stages, which takes place in the normal egg, fails to occur, and in consequence the embryo develops higher up on the egg.

Bryn Mawr College, March 21, 1904.

Zusammenfassung.

1) Die Froscheier, bei denen die erste Furche das Material so ungleich teilt, wie Fig. 1 und 3 zeigen, können einem normalen Embryo zum Ursprung dienen.

Unregelmäßige Teilung, wie in Fig. 6 und 7 und in Fig. 8 und 9, beruht wohl auf Polyspermie.

Eine Analyse der Resultate von MORGAN und BORING führt zu der Hypothese, daß die ausnahmsweisen Fälle (etwa $8^1/_2\%$), in welchen die erste Teilungsebene rechtwinklig zur Mittelebene des grauen Feldes steht, auf einer sekundären Rotation des Eiinhalts beruht, welche die Spindel in eine neue Lage bringt. Die Medianebene des Froschembryos scheint durch die Symmetrieebene im Protoplasma im voraus gegeben zu sein und kann in diesen Fällen entweder mit der ersten Furchungsebene oder mit der Medianebene des grauen Feldes zusammenfallen, je nachdem der eine oder andre Einfluß der stärkere ist, d. h. je nach der größeren Potenz der einen oder der andern Symmetrieebene.

Die Stellung der Spindel in der Zelle scheint durch Protoplasmabewegungen bestimmt zu werden, und diese können wiederum das Ergebnis verschiedener innerer und äußerer Bedingungen sein. Es ist unwahrscheinlich, daß die Ursache der Protoplasmabewegungen für alle Arten von Zellen dieselbe ist.

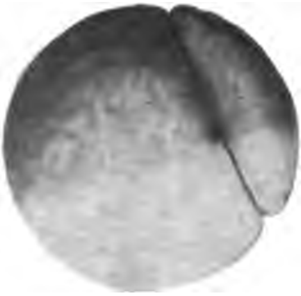
2) Die augenscheinlichen Beweise zugunsten der Annahme, daß die Schwerkraft die Lage des grauen Feldes bestimmt und somit auch die Lage der Medianebene des Froschembryos, schließt die Möglichkeit nicht aus, daß nach Ausschaltung der Schwerkraft (wie bei KATHARINER [1903] und meinen eignen Versuchen [1903]) die Medianebene des Embryos durch einige andre Faktoren bestimmt werden kann.

3) Die in Fig. 16 abgebildeten Halbembryonen wurden durch Anstechen einer der beiden ersten Blastomeren vor Vollendung der ersten Furche erhalten. Die Diagramme Fig. 13, 14, 15 zeigen die Bildungsweise eines derartigen Embryo (Querschnitte *G*, *H*, *I*). Der Kopf des Embryo ist nahezu eine Ganzbildung, die nicht etwa durch nachträgliche Komplettierung einer ursprünglich halben Kopfanlage an deren medialer Seite zustande kam, vielmehr wurde der Kopf von Anfang an nahezu symmetrisch in dem nach vorn von der ersten Lage der dorsalen Blastoporuslippe gelegenen Material angelegt.

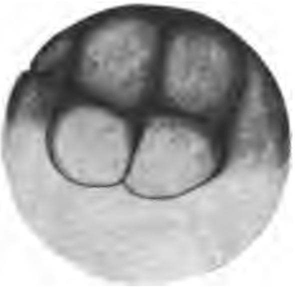
Eine Analyse der die Entstehung eines Halbembryo beherrschenden Bedingungen führt zu der Ansicht, daß, wenn ein solcher Halbembryo zuerst gebildet wird, das unbeschädigte Material der andern Seite nicht zur Hervorbringung der fehlenden Hälfte verwendet werden kann, ohne daß es gleichfalls zuerst den regulären Gastrulationsprozeß zu der Zeit durchlaufen hat, in der die unverletzte Hälfte gastruliert hat.

4) Bisweilen findet man Zwergeier beim Frosch, deren Volumen nicht größer sein kann als das halbe der normalen Eier. Das Volumen dieser Zwergeier entspricht also dem von einer der beiden ersten Blastomeren. Aus ihnen entstehen normale Zwergembryonen. Die Zellen in diesen Zwergembryonen sind kleiner als die in Embryonen ganzer Größe, ein Anzeichen, daß die kleinen Eier dieselbe Anzahl von Teilungen zu durchlaufen haben, wie die normalen Eier. Die isolierte Blastomere der Seeigeleier produziert dagegen nur halb so viele Zellen, wie das ganze Ei.

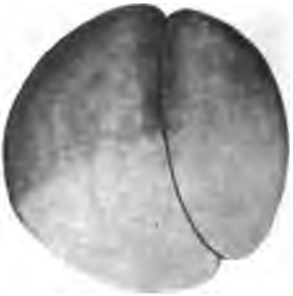
5) Extreme Formen von Spina bifida — Ringembryonen — zeigen, daß die Medullarplatte bis nahe an ihr vorderes Ende geteilt sein kann. Es würde ein Trugschluß sein, deshalb anzunehmen, daß im normalen Embryo die dorsale Blastoporuslippe nahe am vorderen Ende des Gehirns beginnt und daß der Rest des Embryo durch Concrescenz entsteht. Dem Unterschied der beiden Fälle kann durch die Annahme Rechnung getragen werden, daß bei den Ringembryonen das Herabwachsen des Materials in den letzten Furchungsstadien ausfällt, welches bei normalen Eiern stattfindet, so daß bei jenen der Embryo höher oben am Ei sich entwickelt.



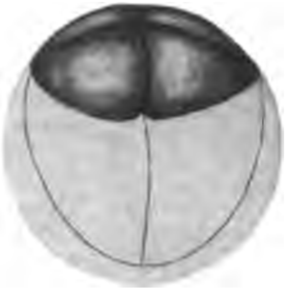
1



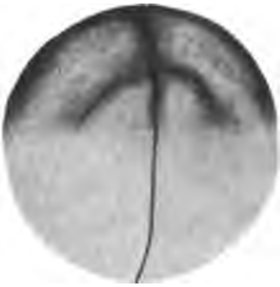
2



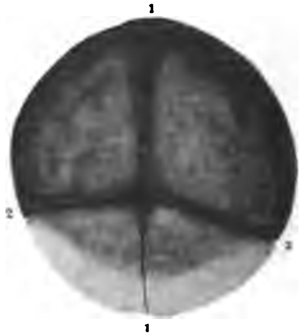
3



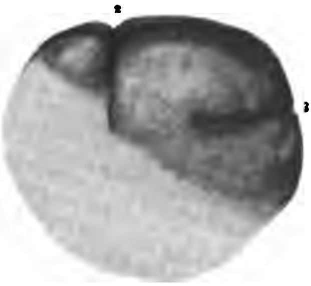
4



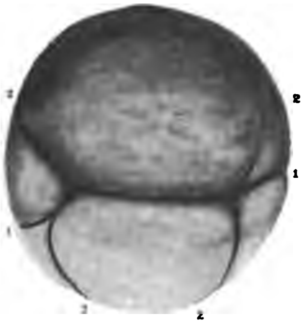
5



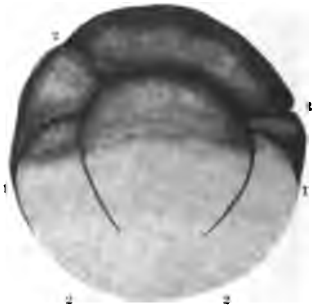
6



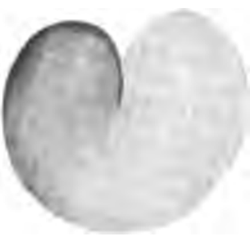
7

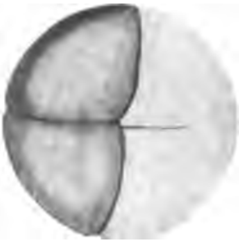


8

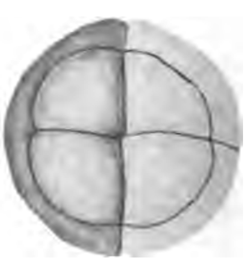


9



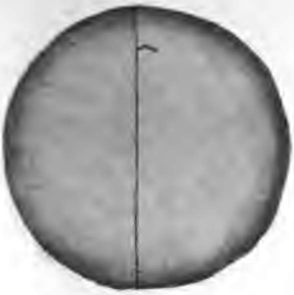


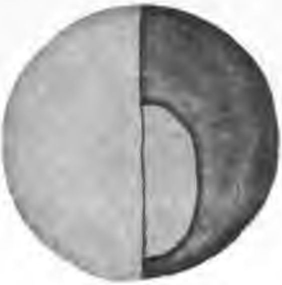
11

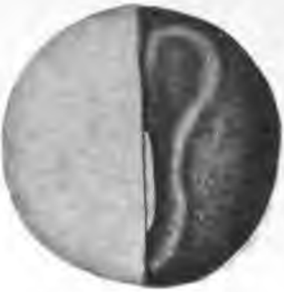


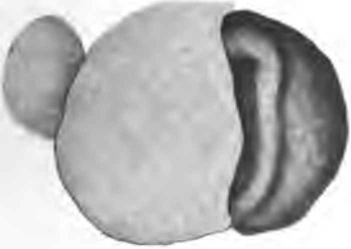
12

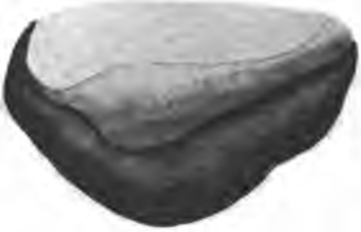
Verlag v Wilhelm Eng

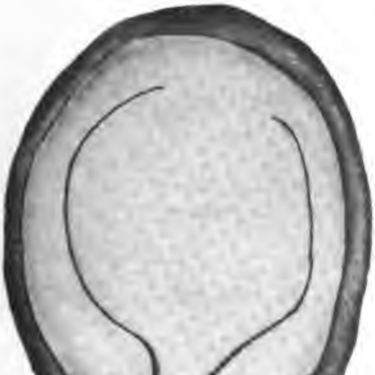

13

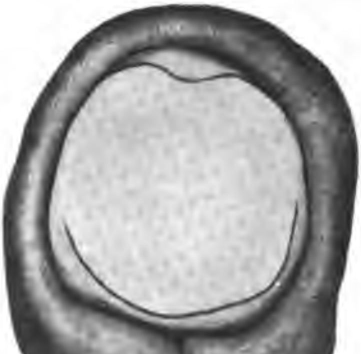

14

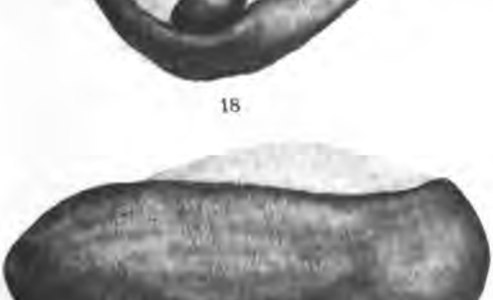

15

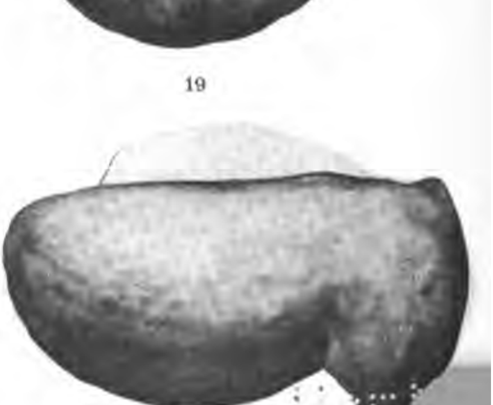

16


17


18

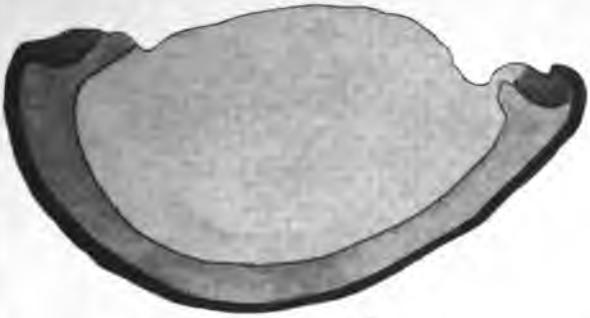

19


20

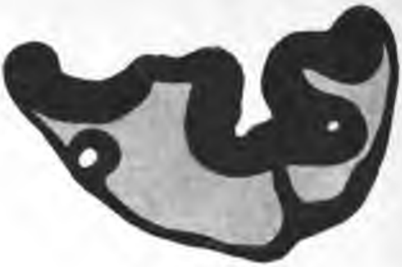

21

elmann in Leipzig

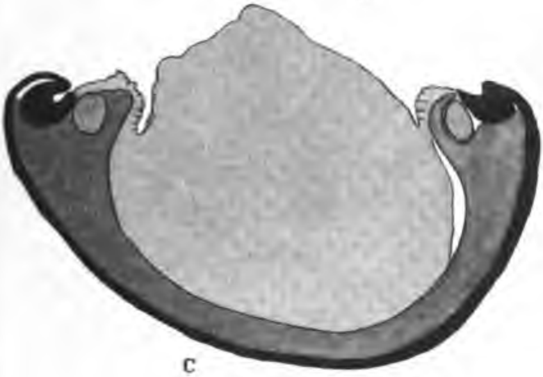
[illegible]



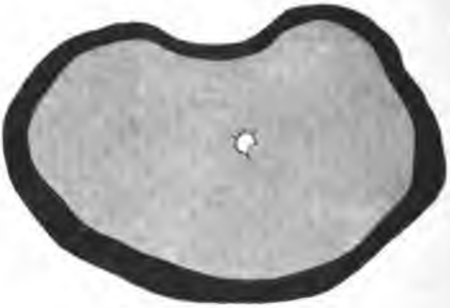
A



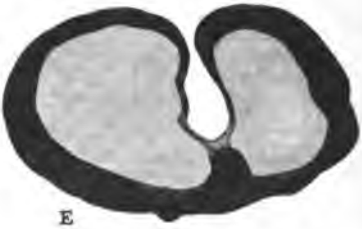
B



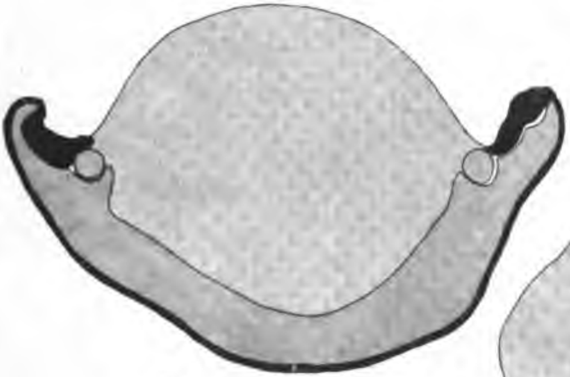
C



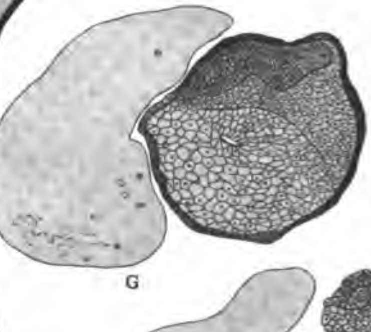
D



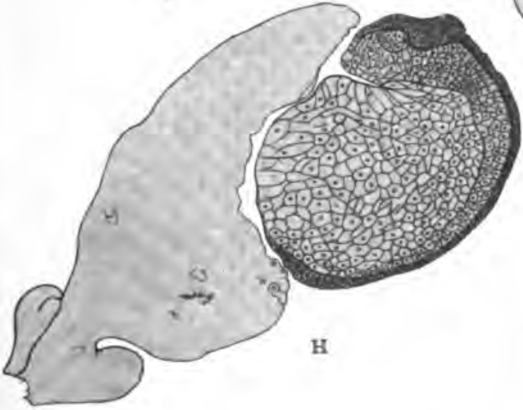
E



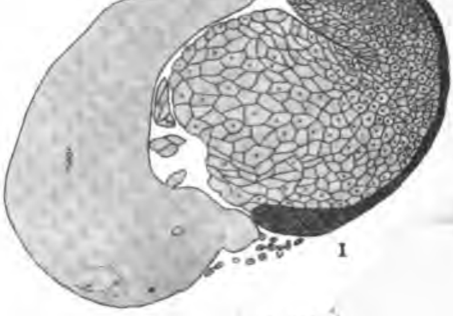
F



G



H



I

Verlag v Wilhelm Engelmann in Leipzig.

Photolithogr. v H. F

Results of Injuries to the Blastopore Region of the Frog's Embryo.

By

Anne Hampton Todd.

With Plates XXIX and XXX and 20 figures in text.

Eingegangen am 25. April 1904.

The principal object of the following experiments was to determine to what extent a known injury to the frog's egg causes it to depart from the normal course of its development and how, after recovery from the effect of the injury, the return to the normal form is accomplished. The experiments were made on the egg of *Rana palustris* and consisted mainly in injuring definite regions along the blastoporic rim and noting the effect on the subsequent development. A number of recent workers have assumed that no conclusion in regard to normal development can be drawn from the results of experiments that injure the egg and it was the purpose of my work to examine more carefully the pretensions of this point of view.

The operation was carried out by puncturing the dorsal lip of the early gastrula. The cells of the lip are killed; but when the needle is heated enough to destroy the lip, so much of the surrounding tissue is also injured that development is much retarded and is often prevented altogether. Eggs treated in such a manner rarely lived. I therefore found it more expedient to remove the dorsal lip with a fine cold needle or with very small scissors. In order to watch the eggs more closely I placed them in watch crystals which I had previously lined with paraffine, fastened each separate egg by its membrane with spun glass or needles, and marked its orientation on the paraffine. In this way I could follow more accurately the formation of the blastopore and the development of the embryo. The eggs were preserved in 3% solution of formaldehyde and the sections were stained with borax carmine and Lyons blue.

The work was carried on in the laboratory of Bryn Mawr College under the direction of Prof. T. H. MORGAN to whom I wish to express my gratitude for his assistance and criticism.

The experiments will be considered under four heads. In the first set the dorsal lip of the blastopore was destroyed in order to determine its importance in the formation and closure of the rest of the blastopore and in the subsequent formation of the embryo. In the second set the eggs were punctured in the equatorial region at graded distances from the dorsal lip, in order to determine whether the active growing area was or was not confined to the region immediately surrounding the dorsal lip. In the third set I produced a small exovate in the centre of the yolk, after the blastopore had become circular, in order to determine whether the blastopore in its later stages was formed by an equal or unequal growth of ventral, lateral and dorsal lips. And in the last set of experiments I destroyed the left half of the dorsal lip to see if the other, the right half, would then produce more than half an embryo. MORGAN had found that eggs, which had been kept in a centrifugal machine for several hours, often showed on one side a development of more than half an embryo; and as I had occasionally found that the destruction of the whole dorsal lip leads to the production of similar embryos I hoped by removing the left side of the dorsal lip to cause the right side to develop more than half an embryo.

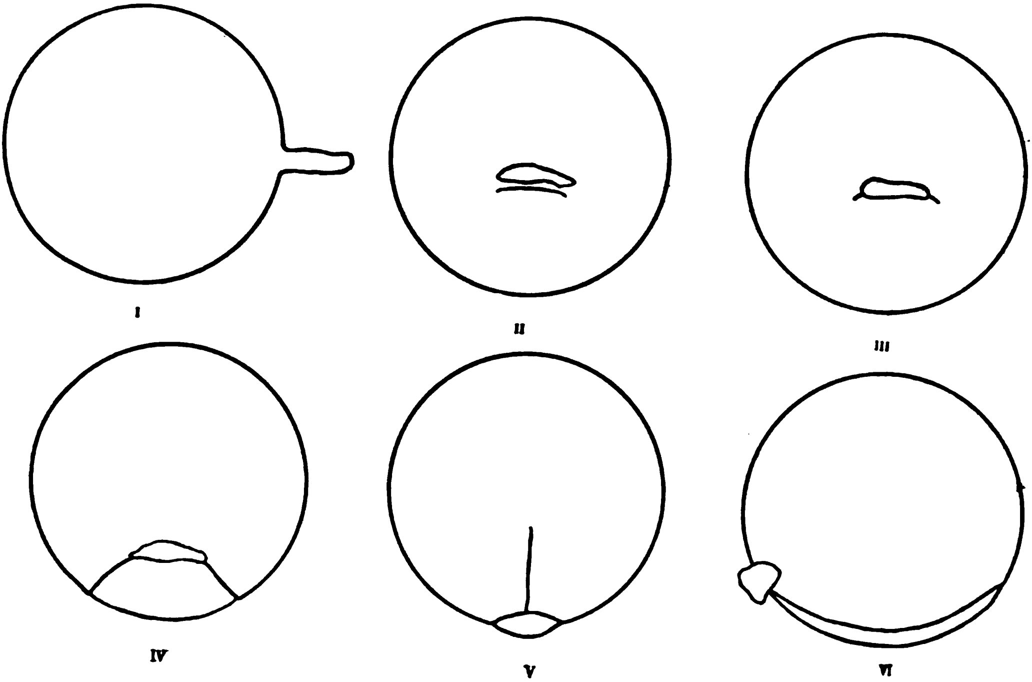
Experiment I.

The dorsal lip of the blastopore was stuck with a cold needle, and as the needle was withdrawn the material of the dorsal lip was extruded as an exovate. According to the size of the exovate thus formed the subsequent development is affected.

A. Method of blastopore closure.

From one to four hours after the operation, the pigment in the cells spreads further and further down, though as yet no definite blastopore rim is formed, and the proportion of light cells to dark steadily decreases. When the blastopore rim reappears it forms lower on the yolk surface and the exovate has been carried down with it; just how I do not at present understand. If the injury to the egg has been slight the ectoderm at either side grows over the region of injury, i. e. over the slight protrusion caused by the injury, and the line of meeting of the ectoderm (Fig. V) is marked on the surface by a seam. The seam increases in length as the blastopore closes. The extent that the blastopore has travelled is thus marked on the surface and the region overgrown by the dorsal lip is shorter in the injured than in the normal egg. The new dorsal lip forms lower on the yolk hemisphere and the blastopore closes just beyond the lower pole. Consequently when the embryo forms it is shorter and its ventral aspect is disproportionately large.

Figs. I—VI.



In other eggs (Fig. II) a new dorsal lip may form below the exovate and lower than the original lip. The lateral and ventral lips, in such cases, grow down normally and the blastopore closes. The exovate is soon detached. Normal embryos result.

The exovate in other eggs (Figs. III and IV) is carried over the yolk surface as the lateral lips are moving towards each other, and prevents the blastopore from closing entirely. The exovate is never a fixed point. By actual observation its progress can be studied.

If the exovate is large the blastopore never closes completely. At times, instead of closing in from all sides either the lateral lips move faster or the ventral lip is held back, and a very long narrow yolk plug is formed (Fig. VI). The medullary folds appear along its margin. Such an embryo soon dies.

The blastopore may progress over the yolk surface in any one of these ways. By the temporary destruction of the dorsal lip its progress is arrested and consequently the dorsal lip never travels as far as in the normal egg, i. e. the blastopore is reduced by a nearly equal growth of dorsal, ventral and lateral lips and closes in the median yolk region. To this extent the injured egg differs from the normal egg.

B. The notochord.

The destruction of the dorsal lip delays or even prevents the growing together of the lateral lips, and in consequence the union of the material from the sides out of which the notochord is formed. In the normal egg the notochord appears about the time the medullary folds are beginning to roll in. On the other hand, in those eggs in which the dorsal lip is destroyed the notochord at the same period shows no signs of separation. In some eggs in which the nervous system has even rolled in and formed a tube no notochord is present. Just why its appearance should be delayed longer than the formation of the nervous system is not clear. In eggs that have been punctured the notochord seems to be derived from the same material as in the normal egg. In some eggs in which the presence of a yolk plug has caused the embryo to develop as separate halves (Figs. 28 and 29) the notochord is formed within the mesodermal sheet of cells; while in others it lies more superficially, near the union of the endoderm and ectoderm cells. The cells at the surface as in Figs. 30, 31 and 32 are sometimes drawn into the notochord. In the embryos with large yolk plugs in the posterior region the posterior part of the embryo is divided into right and left halves. The notochord divides on each side of the yolk plug to form two smaller. cords. Occasionally two notochords, normal in size, are present in one embryo and have no connection with each other throughout their length. In those eggs in which there was earlier present a yolk plug in the posterior region (which later is overgrown) one nervous system is present and the archenteron extends from side to side indicating its former double origin; but the two notochords still persist.

C. Later development.

The peculiarities of later development can best be followed by the consideration of individual eggs.

Egg 1.

The blastopore in this egg (Fig. 1) closed in, leaving a small sized yolk plug. When the medullary folds rolled in the yolk plug was overgrown and its position marked on the surface by a small dent in the posterior third of the nervous system. This embryo shows in sections but one nervous system which posteriorly is undersized and deeply imbedded in mesoderm. Two notochords are present (Fig. 13) and the archenteron is arched in this region. It is difficult to tell whether the nervous system which persisted has come from the union of both sides, or whether it is the nervous system of one side only, — the one remaining, the other disappearing: but as the nervous system in the median and anterior region was of normal size (Fig. 12) I think that the smallness of the cord posteriorly was due to the pressure of the mesodermal thickening on the cord as well as to its formation from the union of two small cords and not to its origin from one side. I can bring forward no direct proof for this conclusion. My argument rests on a knowledge of the conditions found in similar eggs of the same age and younger. The yolk plug causes at times a division of the nervous system and one half only continues to grow as in egg 2. Now if in such an egg the yolk plug were later overgrown, the nervous system ought to be smaller and deeply imbedded in mesoderm in that region and the archenteron ought to be arched and one notochord only should be present, as the material of one half has remained undeveloped. Since two notochords are present in the egg we are considering, it is probable that for a short distance two nervous systems were at first present which, after the overgrowth of the yolk plug, have grown together.

Egg 2.

In puncturing this egg (Fig. 2) the withdrawal of the needle caused the material to be extruded unevenly and so produced an irregular shaped gastrula. The blastopore lips closed in partially. When the medullary folds formed, the yolk plug was carried back and partly overgrown. At the end of two days when the embryo was killed there were present on the surface in the posterior region two yolk plugs. The development in the anterior part of the embryo is fairly normal. The brain and the anterior cord are fully developed but from the middle of the embryo (Fig. 14) posteriorly the cord decreases in size and is covered with mesoderm. In the tail there are present two yolk masses (Fig. 15) — an anterior one, smaller and superficial, and a posterior one, larger which is the true yolk plug. The cells of the anterior yolk mass are cut off from the yolk cells inside. Beneath the plug is a small nervous system imbedded in mesoderm, and a large notochord. The archenteron still runs from side to side and finally opens out on the surface in the region of the second yolk plug just beyond the end of the nervous system. While the original yolk plug was being carried back, probably the cells along its margin closed at some middle point dividing the yolk plug anteriorly and posteriorly. The sides of the nervous system rolled in and closed beneath the anterior yolk mass.

Egg 3.

When the medullary folds formed, the yolk plug was found to lie in the anterior middle part of the dorsal surface of the embryo (Fig. 3). The two halves of the nervous system formed separately. The sections show that in the anterior region (Fig. 16) the nervous system is normal in size and from it the eyes developed as outgrowths. The region is beyond the area injured by the destruction of the dorsal lip and consequently the development is from both sides of the embryo. The destruction of the dorsal lip caused a separate development of the embryo posterior to it. Behind the right eye a second nerve tube is formed (Fig. 17). This second nerve tube runs a short distance by itself before it joins the main nervous system of the embryo (Fig. 18). The walls of the first and second nerve cords are continuous, though the canals are not quite united. At the very anterior end the archenteron has a more central position. It soon moves upward and to the right side and finally comes to the surface between the two nerve cords (Fig. 19). The archenteron is arched and moves from its central position to one nearer the surface sometime before it opens out. The two nervous systems had a separate origin. The study of the sections agrees, therefore, with observations I made on the living egg, — namely, that the yolk plug is carried backwards during the growth of the medullary folds and prevents a union of the two halves of the embryo. The notochord of each half arose independently, and the two never connected. The material of one side of this embryo gave rise to a nervous system having nearly a whole form, although reduced in size. In those embryos in which the presence of the yolk plug causes a division of the material posterior to it the tail knobs are formed separately and its halves never united. When the yolk plug has been entirely overgrown the material of the tail is often rearranged, and the tail grows upwards at right angles to the main axis of the body or even curls back on the embryo.

Egg 4.

In injuring this egg (Fig. 4), when the needle was withdrawn more material than usual was extruded and the blastopore never closed altogether. The head of the embryo is much shortened and twisted, and there is a large yolk plug in the posterior region. The sections show that the brain and eyes are absent. The most anterior sections (Fig. 20) show a single nerve tube, continuous with the left cord, at the sides of which the ear pits are forming. Sections a little further back (Fig. 21) show the anterior end of the right cord and still further back (Fig. 22) the right and left cords come together, their walls touching but not fusing. Almost immediately the two separate again and the yolk plug appears between them (Fig. 23). The left nerve tube runs only a short distance. It begins to disappear as soon as it reaches the yolk plug and all that remains in the posterior region is an ectodermal thickening. There is no notochord belonging to the left side. On the right side the outer wall of the nerve cord is thick while the wall next to the archenteron is thin, indicating that it is not a »whole« nervous system reduced in size, but a half nerve tube. The notochord is present on the right side. The archenteron runs from one side of the egg to the other, even in the anterior end. Here as in Egg 3 the yolk plug has caused a separation of material posterior to it.

Egg 5.

In this egg (Fig. 5) it was observed that the blastopore did not close in entirely and the yolk plug was still present when the medullary folds were rolling in. It was later overgrown and its position marked on the surface by a thickening on either side of the tail. When the sections of this embryo were studied they showed apparently a very one-sided development. In the very anterior end (Fig. 24) the nervous system is normal size and the notochord lies on the extreme left side. In the median region (Fig. 25) the cord is on the left, is imbedded in mesoderm, and is smaller. The canal is nearer the right wall than the left. The mesoderm is much developed on the left side, whereas on the right there is almost none. The archenteron lies to the left of the centre. In the posterior region (Fig. 26) the development is even more confined to the left side. There is a break in the ectoderm and mesoderm just to the right of the centre showing where the yolk plug was more recently overgrown. The size and position of the nervous system, notochord, archenteron, and mesoderm in the tail indicate that the development is strictly one-sided. Except for the position of the notochord the relation of the parts of the embryo in the head is normal; that is, development has taken place on both sides. From the history of the embryo and from a study of the sections I conclude that the development in the anterior region is from both sides and that in the median and tail region it is confined to the left side. In other eggs killed younger than this one as Egg 2 for example I often found that a yolk plug caused a division of the material and that the nervous system posterior to it developed only on one side. Egg 5 is probably an older stage of such a case.

Egg 6.

This ring embryo (Fig. 6) is chiefly interesting because more than half an embryo has developed from one side. On the right side the nervous system and the notochord have formed and mesoderm is present on both sides (Fig. 27). The development on the left side is a half embryo.

Egg 7.

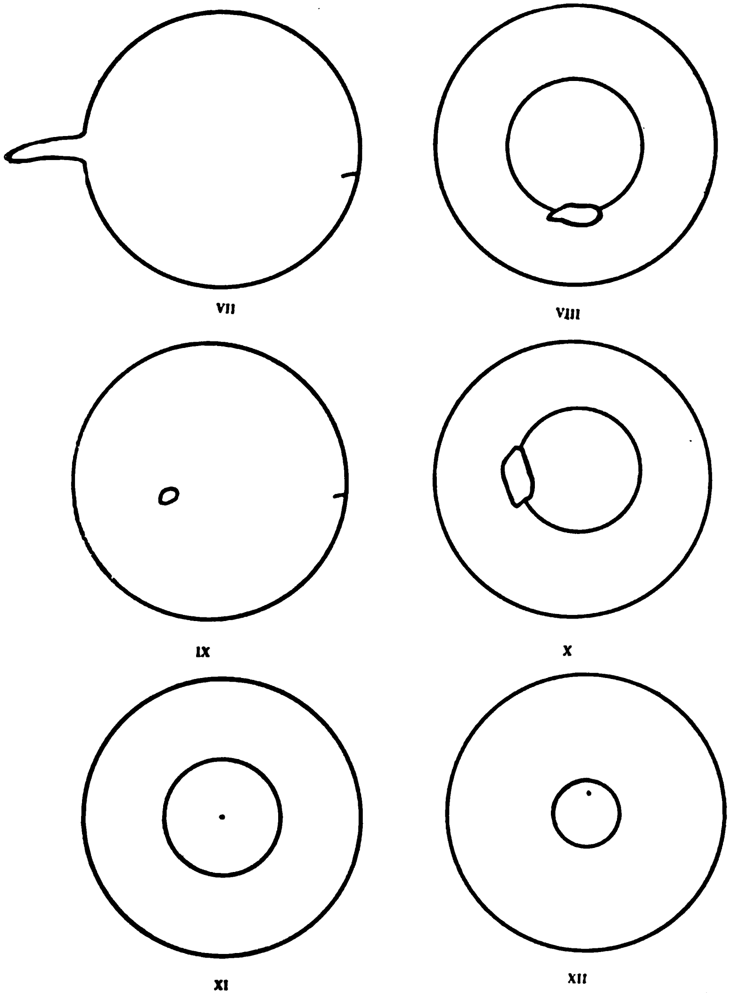
The development of the left side, in this embryo (Fig. 7) is more than half. The notochord is present with mesoderm on both sides and the nervous system above it (Fig. 29). The nerve cord in the right side, is represented by an ectodermal thickening.

I found other embryos similar to these two but showing nothing essentially new.

Experiment II.

Twenty eggs were stuck (Fig. VII) on the first appearance of the dorsal lip at a point nearly opposite to it on the white hemisphere about 150 degrees away. When the ventral lip formed (Fig. VIII) the exovate was found to be on its edge although the injury had been made higher up on the egg. In some cases, when the exovate was small, it was overgrown; but usually the blastopore did not close in completely owing to the presence of the exovate at the ventral lip. The destruction of the region of the ventral lip leads to the appearance of a dorsal yolk plug in the posterior region of the embryo.

Figs. VII—XII.


VII VIII IX X XI XII

Experiment III.

Twenty eggs were punctured on the left side (Fig. IX) in the region from which the lateral lips of the blastopore develop half way between the dorsal and ventral lips at the time when the dorsal lip appears. The exovate (Fig. X) retarded the closing in of the blastopore. In those eggs in which the exovate still remained it was attached to the lateral lips.

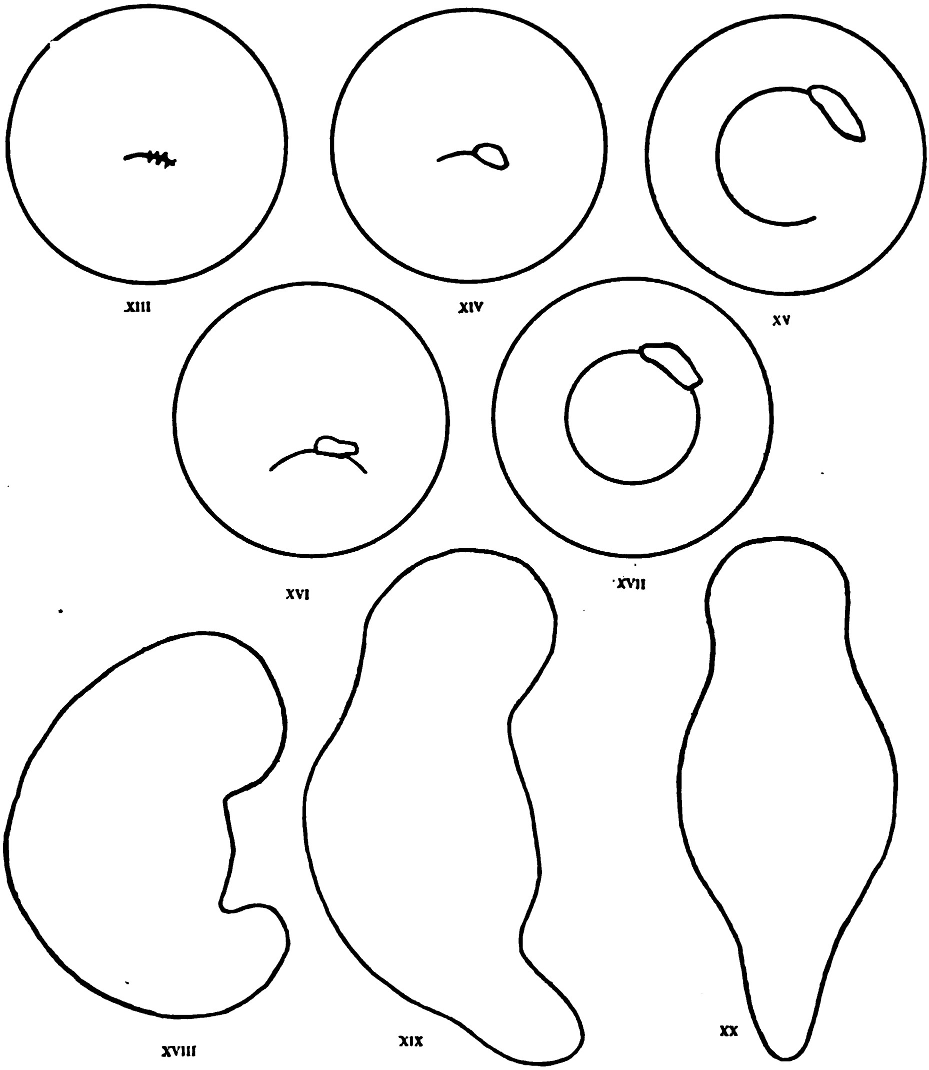
Similar experiments were carried out on other eggs puncturing them at different points along the lateral line between the dorsal and ventral lips. Here too the exovate was attached to the lateral lips of the blastopore and retarded its closure.

From this experiment and experiment I and II, we must conclude that there is an active growing area extending around the equator of the egg from which the sides of the embryo are formed. An injury anywhere in the lower hemisphere usually delays the closure of the blastopore.

Experiment IV.

In injuring the dorsal lip of the blastopore an exovate is produced in the region of the smallest cells and therefore in the most actively growing and moving area. Such an exovate cannot be used as a definite point for marking the relative growth and closure of the blastopore. On the other hand the yolk cells of the lower pole are large and comparatively immovable and therefore an exovate in this region can be used to better advantage as a point to indicate the movement of the blastopore lips. With a very fine needle I punctured 100 eggs in the median yolk region just after the blastopore had become circular (Fig. XI). Out of this lot I selected 30 eggs in which I considered the exovate to be equidistant from all sides of the blastopore. After puncturing, each egg was fastened by its membranes with spun glass and needles to paraffine in the bottom of a watch glass and a mark made on the paraffine corresponding to the position of the dorsal lip on the egg. In the early stages of the circular blastopore the dorsal lip is marked by a deeper indentation which can easily be detected with a hand lens; but to avoid any mistake I kept many of the eggs orientated until the medullary folds appeared — thus making certain the location of the dorsal lip. In every case I found that at the end of two hours the dorsal lip was one third nearer the exovate than either the lateral or ventral lips (Fig. XII). And in three to four hours the dorsal lip had approached the exovate and had overgrown it. During the fourth hour the dorsal lip pushed on beyond the yolk pole; the dorsal lip had travelled over about two thirds of the yolk area. This experiment proves, first, that the dorsal lip of the blastopore progresses to a point beyond the yolk pole, and secondly, that from the time the blastopore becomes nearly circular the dorsal lip moves about one third faster than do the ventral or lateral lips. If the dorsal lip moves faster in late stages, it is probable that in the earlier stages it also moves more rapidly. In fact, MORGAN's experiments go to show that this is the case.

Figs. XIII—XX.



XIII XIV XV XVI XVII XVIII XIX XX

Experiment V.

The left half of the dorsal lip was stuck on its first appearance (Fig. XIII) and an exovate was formed. The right side of the dorsal lip extends around the egg and the ventral lip often forms before the left part of the dorsal lip appears (Fips. XIV and XV), and when it does the new left side of the dorsal lip is formed beyond and under the exovate i. e. (more in the yolk area than the first position of the left side of the lip). The destruction of the left half of the dorsal lip leads, in the main, to the same result as does the destruction of the dorsal lip. In some eggs the exovate is overgrown and the meeting of the ectoderm over it is indicated on the surface by a crooked line. Normal embryos result. The exovate, in other eggs (Fig. XVII), was so large as to retard the closure of the blastopore. A protruding yolk plug is formed in the centre of the yolk hemisphere with an exovate on the left side of the yolk plug. When the medullary folds appear in such eggs the yolk plug is found to lie in the middle part of the dorsal surface of the embryo. Many of the embryos in this experiment bend over to the left side (Figs. XVIII, XIX and XX); but in the course of two or three days such an embryo straightens itself. The bending was probably due to the material lost in removing the left part of the dorsal lip and also to the subsequent slower growth of the left half of the embryo.

I had hoped by injuring the left part of the dorsal lip to be able to produce embryos in which the right side would produce more than a half structure, such as I had sometimes obtained in destroying the whole dorsal lip. No such results were obtained. In all cases the right side produced its half embryo.

Review.

IKEDA in his recent paper on ›The Mode of Blastopore Closure and Position of the Embryonic Body‹, has considered many of the same questions that I have examined in this paper, but he has reached quite different conclusions. He claims that his observations show that the normal course of development is not followed in eggs that have been punctured. IKEDA maintains that:

1) The exovate caused by puncturing the egg is a fixed point.

2) There is an overgrowth of the ventral and lateral lips to meet the exovate when the dorsal lip is destroyed.

3) On injuring the egg in one or more places at or below the level of the dorsal lip, the blastopore closes at the point of greatest injury.

4) Hence the location of the embryonic body may be either on the upper or the lower hemisphere or partly on both.

5) And therefore the method of normal development cannot be determined from the study of embryos that have been injured.

All these conclusions are based on the assumption that the exovate is a fixed point. If it can be shown that the exovate is not a fixed point, IKEDA's conclusions fall to the ground. His work was done on another species of frog and it is possible that a different mode of development may be followed in that species, but I do not think it is probable. In those eggs in which I produced an exovate at any point along the equator I found that as the blastoporic rim moves over the yolk surface the exovate must either move with it or become detached. In either case the exovate ceases to be a fixed point. If the injured dorsal lip together with the exovate moves backward over the lower hemisphere, as I have shown is the case, there is no need to assume that the reduction of the blastopore is brought about by the forward growth of the ventral and lateral lips, as IKEDA claims. The same explanation can be applied to another proposition that IKEDA makes, — namely, that the blastopore always closes at the point of greatest injury. When the exovate lies along the equator of the egg it is later carried away by the edge of the blastopore which is growing down over the surface of the egg. Hence while the blastopore seems to close at the point of greatest injury, in reality the exovate has been carried down to the point at which the blastopore closes. I conclude from the evidence of my experiments, that the exovate is not a fixed point; and if there is no more than normal forward growth of the ventral and lateral lips over the yolk; and if the blastopore does not close at the point of greatest injury, then the position of the embryo on the egg is not indifferently on the upper or lower hemisphere surface, as IKEDA maintains, but confined mainly to the lower hemisphere and hence normal development can be at least partially determined from the study of injured eggs. It is true that if a very large exovate is formed the contents of the egg may be drawn over towards the region of injury and the closing of the blastopore interfered with and the development soon brought to a standstill. Extensive injuries of this kind cannot however fairly be brought forward as evidence to show that smaller injuries to the egg cannot be used with caution to furnish evidence as to the normal development.

Summary.

When the dorsal lip of the blastopore is stuck with a cold needle so that its material protrudes, the following changes occur:

a) A new dorsal lip may form below the original lip and grow slowly and normally over the yolk; or,

b) When the median part of the dorsal lip does not reappear and an exovate is formed there, the lateral lips move down over the yolk surface carrying the exovate along with them; or,

c) A small exovate is sometimes overgrown by the sides of the blastopore lips and the point of meeting of the ectoderm over the exovate is marked by a seam. The seam increases in length as the blastopore moves down. The distance which the dorsal lip has travelled is thus marked on the surface.

d) Owing to the retarding effect of the exovate the blastopore closes in at the lower pole of the egg, rather than further on as in the normal egg.

e) The embryo develops mainly over the lower hemisphere; the head forming in and just beyond the region in which the dorsal lip was first seen, and the tail appearing at a point on the lower hemisphere nearly opposite (Roux).

f) The notochord develops at a later period than in the normal embryo.

g) When the medullary folds roll in a yolk plug is usually present in the middle of the dorsal surface of the embryo (Roux).

h) While the medullary tube is forming the yolk plug is generally carried back and overgrown from the sides.

i) The presence of the yolk plug delays or prevents the union of the halves of the embryo posterior to it.

j) In some embryos in which the yolk plug was early overgrown the separate parts of the nervous system unite but the resulting cord is always small and imbedded in mesoderm.

k) The presence of a yolk plug may cause a separate development of the halves of the embryo both anterior and posterior to the plug. Just in front of the yolk plug the walls of the both nervous systems may fuse though the canals are never continuous. The notochords are separate in origin and never unite.

l) Only one half of the embryo posterior to the yolk plug may continue to develop.

m) In some few embryos the material of one side only may give rise to a nervous system having nearly a whole form, with the notochord just beneath, and mesoderm on either side of the notochord; i. e. an embryo that is more than a half on one side.

It was found that when exovates were produced (at the time of the first appearance of the dorsal lip of the blastopore) just below the equator of the egg in the regions where the ventral or lateral lips would later form that:

a) When the blastopore appears the exovate is attached to the edge of the lip, which shows that the sides of the embryo are formed from the material that lies just below the equator of the egg.

When an exovate was produced at the lower pole in the yolk just after the blastopore had become circular it was found that:

a) From the time the blastopore becomes circular the dorsal lip moves $^1/_3$ faster than do the ventral and lateral lips.

b) The blastopore closes in the normal egg at a point beyond the lower pole.

When the left part of the dorsal lip is removed with a cold needle:

a) The right side of the dorsal lip extends around the egg and the ventral lip often appears before the left part of the dorsal lip is formed again.

b) The exovate, in those eggs in which the blastopore does not close completely, lies to the left of the centre.

c) Some of the embryos are bent to the left side, but in the course of two days straighten themselves.

d) When, as in this experiment, one side of the early blastopore is destroyed, more than half an embryo did not develop on the other side.

April, 1904.

Bryn Mawr College, Bryn Mawr, Pennsylvania.

Zusammenfassung.

Sticht man mit einer kalten Nadel in die dorsale Blastoporuslippe so ein, daß ein Vorfall des Bildungsmaterials erfolgt, so kommt es zu folgenden Veränderungen:

a) Eine neue dorsale Lippe kann sich unter der ursprünglichen bilden und sich allmählich in normaler Weise über den Dotter ausbreiten, oder

b) wenn der mediane Teil der dorsalen Lippe nicht wieder erscheint und an ihrer Stelle ein Extraovat entsteht, so rücken die seitlichen Lippen über die Dotteroberfläche herab, indem sie das Extraovat mit sich fortschieben, oder

c) es wird ein kleines Extraovat manchmal von den Blastoporusrändern von der Seite her überwachsen und der Vereinigungspunkt des Ektoderms über dem Extraovat durch eine Naht bezeichnet. Diese Naht wächst mit dem Abwärtsrücken des Blastoporus in die Länge. Dadurch wird der von der dorsalen Lippe zurückgelegte Weg an der Oberfläche markiert.

d) Infolge des verzögernden Einflusses des Extraovats schließt sich der Blastoporus häufiger schon am unteren Eipole, als jenseits desselben, wie beim normalen Ei.

e) Der Embryo entwickelt sich hauptsächlich über die untere Hemisphäre hin: Der Kopf bildet sich in und dicht neben dem Bezirk, wo die dorsale Lippe zuerst auftrat, und der Schwanz erscheint an einem nahezu entgegengesetzt liegenden Punkt der unteren Hemisphäre (Roux).

f) Die Chorda entwickelt sich in einer späteren Periode als beim normalen Embryo.

g) Sind die Medullarwülste angelegt, so zeigt sich ein Dotterpfropf gewöhnlich in der Mitte der Embryorückenfläche (Roux).

h) Bei der Bildung des Medullarrohrs rückt der Dotterpfropf im allgemeinen nach hinten und wird von den Seitenwänden überwachsen.

i) Die Gegenwart des Dotterpfropfs verzögert die Vereinigung der nach hinten von ihm gelegenen Embryohälften.

j) Bei einigen Embryonen, in denen der Dotterpfropf frühzeitig überwachsen wurde, vereinigten sich die getrennten Teile des Nervensystems, aber der sich ergebende Strang ist immer klein, schmal und in Mesoderm eingebettet.

k) Die Gegenwart eines Dotterpfropfs kann getrennte Entwicklung der Embryohälften veranlassen, sowohl vor wie hinter dem Dotterpfropf. Gerade vor dem Dotterpfropf können die Wände der beiden Nervensysteme verschmelzen, aber die Zentralkanäle liegen nie in einer geraden Linie. Die Chordae sind ursprünglich getrennt und vereinigen sich nie.

l) Es kann die eine Hälfte des Embryo, welche hinter dem Dotterpfropf gelegen ist, allein die Entwicklung fortsetzen.

m) In einigen wenigen Embryonen kann das Material nur einer Seite allein ein Nervensystem von annähernder Vollständigkeit hervorbringen, mit der Chorda unmittelbar unter ihm und Mesoderm zu beiden Seiten der Chorda, d. h. einen Embryo, welcher mehr als die Hälfte auf einer Seite darstellt.

Bei der Erzeugung von Extraovaten (zur Zeit des ersten Erscheinens der dorsalen Blastoporuslippe) gerade unterhalb des Eiäquators in der Gegend der späteren unteren und seitlichen Blastoporusränder fand sich folgendes:

a) Beim Erscheinen des Blastoporus bleibt das Extraovat am Rande der

Lippe haften, woraus also hervorgeht, daß die Embryoseiten aus dem unmittelbar unterhalb des Eiäquators liegenden Material gebildet werden.

Bei der Entstehung eines Extraovats am Dotter, am unteren Pol, kurz nach dem Kreisförmigwerden des Blastoporus, wurde folgendes beobachtet:

a) Vom Zeitpunkt des Kreisförmigwerdens des Blastoporus an bewegt sich die dorsale Lippe um ein Drittel schneller als dies die ventrale und die Seitenlippen tun.

b) Der Blastoporus schließt sich beim normalen Ei an einem jenseits des unteren Poles gelegenen Punkt.

Nach Entfernung des links gelegenen Teils der dorsalen Blastoporuslippe mit einer kalten Nadel ergab sich:

a) Die rechte Seite der dorsalen Lippe dehnt sich rund um das Ei aus und die ventrale Lippe zeigt sich öfter, bevor der linke Teil der dorsalen Lippe wieder gebildet ist.

b) Das Extraovat liegt bei den Eiern mit unvollständigem Schluß des Blastoporus links vom Zentrum.

c) Einige von den Embryonen erschienen nach links verbogen, streckten sich aber wieder im Verlauf von 2 Tagen.

d) Wird, wie bei dem vorliegenden Versuch, eine Seite des eben gebildeten Blastoporus zerstört, so entwickelte sich nicht mehr als die Hälfte eines Embryo auf der andern Seite.

Explanation of Plates.

Plates XXIX and XXX.

Figs. 1—11. Surface views of embryos in which the dorsal lip of the blastopore had been earlier removed.

Fig. 1. An embryo in which after the yolk plug was overgrown the nervous system of each side united.

Fig. 2. An embryo in which the nervous system has rolled in and formed beneath the yolk plug.

Fig. 3. An embryo in which the presence of a yolk plug has caused the separate development of each half.

Fig. 4. The anterior part of the embryo (the brain and eyes) are absent. Two nerve cords, separate in origin, are present.

Fig. 5. The nervous system in the posterior region has been derived from the material of one side only.

Figs. 6 and 7. Eggs in which more than half an embryo has developed on one side.

Fig. 8. Posterior to the yolk plug the halves of the embryo have not united into a whole structure.

Figs. 9—11. To show the method of overgrowth of the yolk plug.

Fig. 9. The yolk plug is present in the middle of the dorsal surface of the embryo.

Fig. 10. The yolk plug has become smaller and has been carried further back.

Fig. 11. The yolk plug has been entirely overgrown and its position in the tail marked on the surface by a depression.

Figs. 12 and 13. Sections through the middle and posterior region of egg 1, Fig. 1.

Figs. 14 and 15. Sections through the middle and posterior region of egg 2, Fig. 2.

Figs. 16, 17, 18 and 19. Sections through the anterior and middle part of egg 3, Fig. 3.

Figs. 20, 21, 22 and 23. Sections through the anterior and middle part of egg 4, Fig. 4.

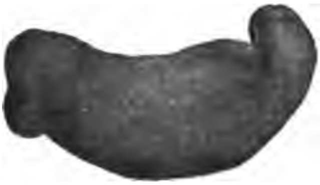
Figs. 24, 25 and 26. Sections through the anterior, middle and posterior region of egg 5, Fig. 5.

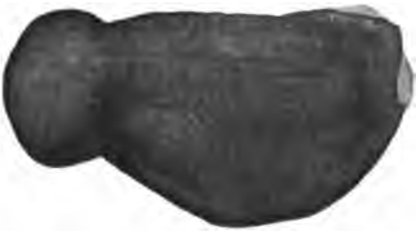
Fig. 27. Section through the middle of egg 6, Fig. 6, showing that more than half an embryo has developed on one side.

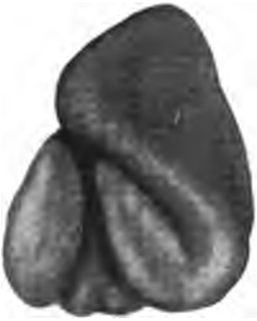
Fig. 28. A part of Fig. 29 enlarged to show the separation of the notochord from mesoderm.

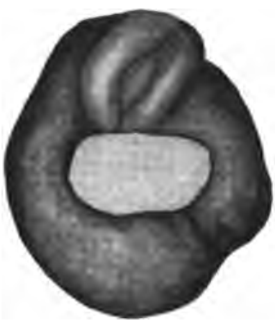
Fig. 29. Section through the middle of egg 7, Fig. 7.

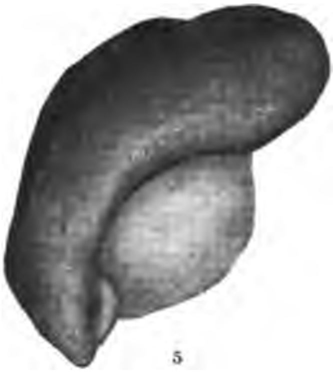
Figs. 30, 31 and 32. Sections through an embryo showing the origin of the notochord from mesodermal and ecto-endodermal cells.

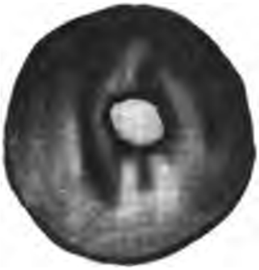

1

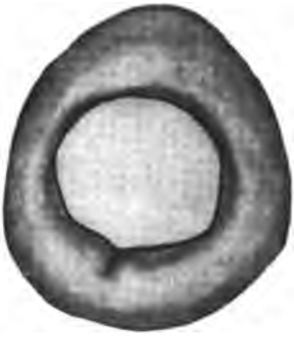

2

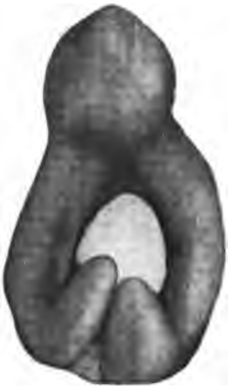

3

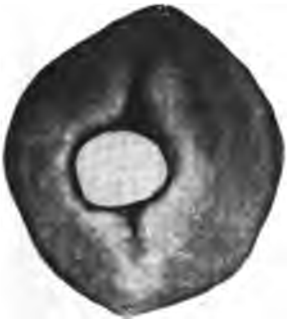

4

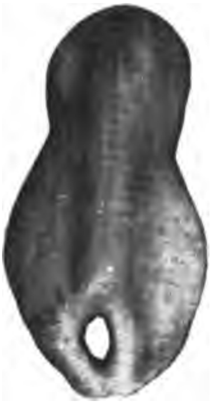

5

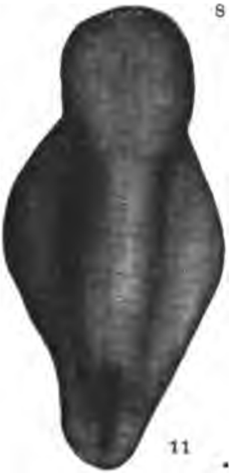

6


7

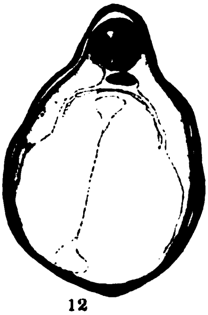

8

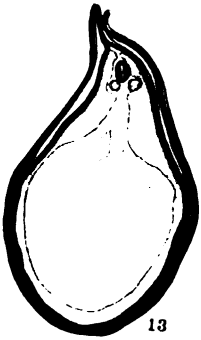

9

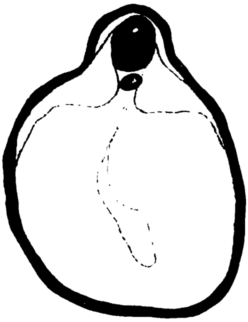

10

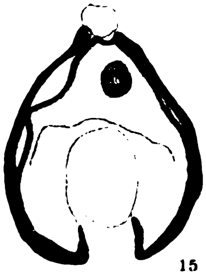

11

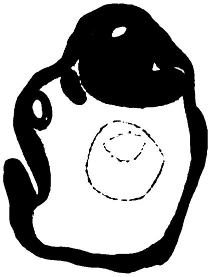
Verlag [illegible] Photolithogr. v. H. F. [illegible]

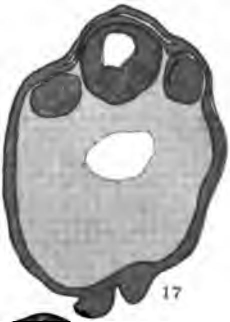

12

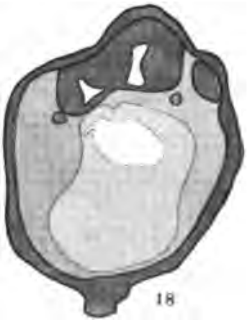

13

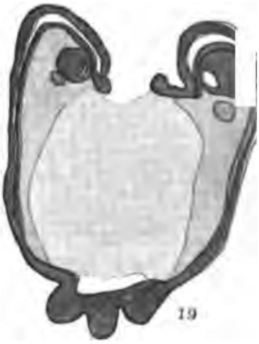

14

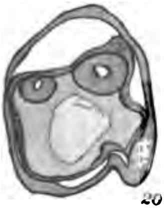

15

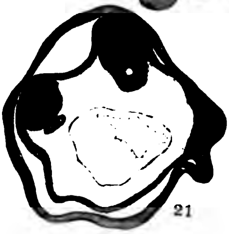

16

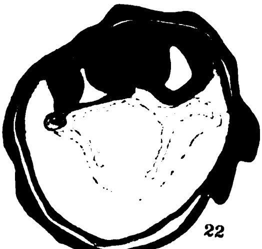

17

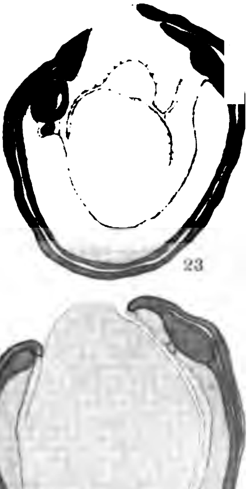

18

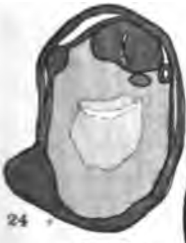

19

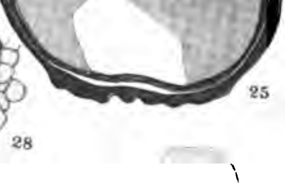

20

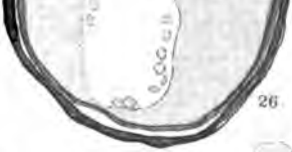

21

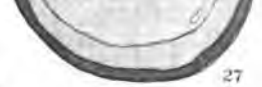

22

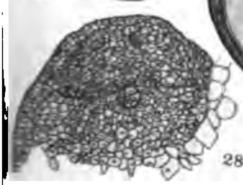

23

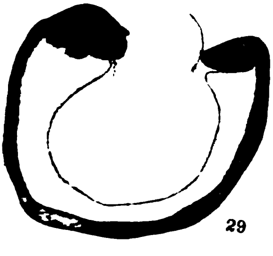

24

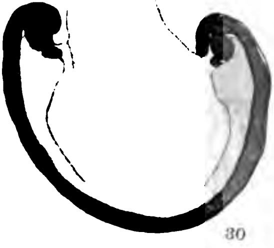

25

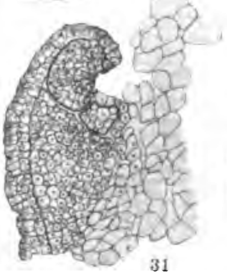

26

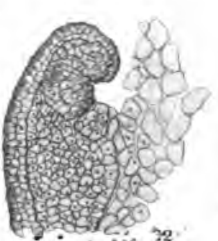

27


28


29


30


31


32

Th
mo
i

w
of
of
tw
th
to
ca
in
wi
on
de

ra
sa
th
ma
lie
of
me
wh
wi

The Relation Between Normal and Abnormal Development (IV), as Determined by Roux's Experiment of Injuring the First Formed Blastomeres of the Frog's Egg.

By

T. H. Morgan and Ellen Torelle.

With Plate XXXIII.

Eingegangen am 25. April 1904.

The object of the experiments described in the following pages was two-fold. In the first place we wished to make an examination of the immediate results of the operation, first carried out by Roux, of injuring with a hot needle one of the first two (or, in some cases, two of the first four) blastomeres of the frog's egg. In the light of the discussion that has taken place in the last few years in regard to the outcome of this operation it seemed worth while to make a careful examination of the eggs soon after they had been injured, in order to discover just what is accomplished by injuring the eggs with a hot needle. We wish to lay more emphasis on this side of our results, although we have also examined the later stages of development as well.

In the second place, we have carried out another series of operations which involves a different set of questions although at the same time the question as to the immediate results of the injury is the same here as in the first case. The presence of the »gray area« makes it possible to determine which of the first formed blastomeres lie on that side of the egg that usually gives rise to the head end of the embryo. By injuring them with a hot needle their development can be delayed, or even prevented, and the question arises whether under these circumstances the two »posterior blastomeres« will give rise to the posterior end of an embryo. Roux has also carried out a few experiments of this kind. He thought it probable that the uninjured part of the egg gives rise to the posterior part of an embryo — a suggestion that HERTWIG has rediculed, although, as will appear, without sufficient justification.

Into the controversies that have arisen over this experiment we have no wish to enter. In a sense the experiment has become a classic one in the study of experimental embryology, for, through it, ROUX was lead to some important generalizations, which (together with the earlier experiments of PFLÜGER on the frog's egg) have been the point of departure for much of the later work.

ROUX's early experiments were made by making a needle quite hot and sticking with it two or three eggs successively. He states that, in this way, while the needle may have been too hot for the first egg, and too cold for the last one, in the second case it may have been at the temperature best suited to kill the injured blastomere but not to injure the other one; or the first egg may have been best in some sets, and the third in others. In this way, it may be true, a larger percentage of half-embryos may be obtained, but this method will not recommend itself when it is necessary to find out what changes the injury brings about in the egg, since it is not possible to tell, even approximately, which eggs have been injured to the same extent, and the subsequent development depends largely on the extent and the kind of injury caused by the hot needle. We have therefore adopted a somewhat different procedure. It was first determined by experiment about how hot the needle must be in order to insure that the injury be as great as possible to one blastomere without injuring the other. If the needle were made hotter than this, both blastomeres were usually killed or injured; if less, the blastomere that is stuck will later continue, often irregularly it is true, to divide. It is difficult, in fact, to prevent segmentation from taking place, though it is often confined to the upper region of the injured blastomere.

The crudeness of this method of killing one of the blastomeres will we believe impress itself on any one who familiarizes himself with this experiment. Compared with the precision with which experiments can be carried out on the eggs of the sea-urchins, ctenophores, cerebratulus, and other marine forms, the experiment is primitive in the extreme. We may anticipate one of our conclusions and state here that we have been much surprised to find how difficult it is to injure, or at least to kill, the nucleus of the injured blastomere. Its small size, and the fact that it lies so deep in the protoplasm, probably accounts in part for its escape from the effects of the heated needle, which is no doubt greatly cooled before it reaches the region of the nucleus. Nevertheless, comparing all our results with those of Roux, we believe that the injury that we have made is as serious, if not more so, than that which he brought about.

In the spring of 1902 one of us operated on over four hundred eggs of *Rana palustris* at the two-, and four-cell stages. The needle was intentionally made very hot and in consequence most of the eggs were completely killed; so that of those that survived it was quite certain that almost the maximum injury that they will stand had been done to them. In the best lots only from four to ten eggs out of each fifty turned out well. All of the eggs were kept under observation for several hours after the operation. It was found that when the needle was thrust into the egg as much as 45 degrees, or more from the top of the first furrow, that the upper part of the injured blastomeres almost invariably split off small cells, showing that the nucleus had not been seriously injured, if injured at all. When the needle was thrust in nearer to the top of the egg, some of the protoplasm of the other blastomere was generally injured although, as stated above, the nucleus nearly always escaped serious injury.

In those cases in which the anterior two of the first four blastomeres were stuck the eggs were separated into two lots. In one lot, a), the injured half had subsequently divided (generally only at the top at first). The posterior lip of the blastopore appeared later on the uninjured side, but its appearance was generally delayed, and usually a pigmented line extended on one or both sides into the region of the injured half, where subsequently a dorsal lip sometimes appeared. When no segmentation took place in the injured cells, lot b), most of the egg died without forming a blastopore. In a few, a posterior lip appeared, but again it seemed that this took place in only those cases (possibly with rare exceptions) in which some evidence of the lateral lips could be detected on the injured half of the egg, showing that this part had not been completely killed.

In a few other experiments the two posterior blastomeres were stuck. If the injury was not too extensive, the anterior uninjured half of the egg produced a dorsal lip (as in the normal egg), which grew downwards towards the injured half. Whether a half anterior embryo would develop in such cases, as Roux claims, or a whole embryo of half size, i. e., one reduced proportionately to the half size of the material, could not be determined definitely. No doubt either result might take place, depending upon whether or not the protoplasm shifted in the uninjured blastomere, and also possibly depending upon the extent of injury done to the injured cells, although this is more uncertain.

Another possibility, which does not seem to have been fully considered heretofore, was also taken into consideration. An attempt was made to see if the results obtained are different if the operation is made at a time when the first cleavage furrow has just appeared, or if made at a time when the cell-wall has divided the contents of the egg into two parts. In the former case it is conceivable that the operation might prevent the further formation of the cleavage-furrow, and at the same time kill one of the first two nuclei, in which case the contents of the egg would remain continuous, so that the injured side might become incorporated into the other half, a »postgeneration« of a rather unique kind. In fact, if sticking the egg before the cleavage plane has passed through prevents the further advance of this furrow, then it must often occur that the interior is continuous from side to side, because as a rule the first furrow is not usually completed until just before the second furrow appears, and it is during this time that the operation would be carried out. It has been shown by one of us that the development of the second spindles goes on during the time that the first furrow is completing itself. They may develop so far that the chromosomes have divided at the equatorial plate by the time that the first furrow has reached the lower pole.

The experiment showed that when one side was stuck before the furrow had gone very far that the furrow completed itself. This might have been anticipated had it been known how seldom the nucleus of the blastomere is killed, and indeed it is not improbable that the division of the protoplasm might have completed itself even if the nucleus had been actually killed[1]. The question of the importance of the cell wall will be later considered from a somewhat different point of view.

---

[1] As in those cases in which a piece of a dividing egg or of a protozoon is cut off and the division completes itself in the non-nucleated piece.

During the spring of 1903 we repeated these experiments and in the autumn and winter about 125 of the best eggs that had been preserved in various stages were sectioned.

In fifty-five eggs, one of two blastomeres were stuck; in seventy five, the two anterior of the first four blastomeres had been operated upon. Some eggs were preserved in formalin; but the greater number were killed in corrosive-acetic and preserved in alcohol. With the exception of about a dozen slides which were stained in DELAFIELD's haematoxylin followed by Orange G, the sections were stained in borax carmine followed by Lyon's blue.

The injured eggs were killed at regular intervals; either immediately after the operation, so that the effects of the injury could be noted; or after one-half, after one, two, three, four, six, seven, or eight hours. None were killed after the eight hour interval until twenty-four hours had elapsed, and after that, eggs were allowed to develop one, two, three, and five days.

The needle was heated before each operation and applied at a point in the periphery as near as possible to the supposed position of the nucleus of the blastomere to be operated upon. Sections show, however, that it is seldom possible to kill the nucleus of the cell that is stuck, and very difficult to prevent injury being done to the protoplasm of the blastomere which is not operated upon. It is also practically impossible by this method to injure two of the four blastomeres exactly in the same way, or to the same extent, so that as will be seen hereafter the subsequent development of eggs, similarly operated upon, may be different. It is therefore impossible to obtain successive stages of development for comparison; i. e. out of serial sections of one hundred and twenty-five eggs, no one series can be used to absolutely determine what has taken place in an earlier or later stage of development in another series.

The results of the operation can be described under two heads:

1) Effects upon the nucleus.
2) Effect upon the protoplasm and yolk.

The nucleus of the injured blastomere is sometimes, although rarely killed. In about 10 per cent of the blastomeres operated upon, there is little doubt that the nucleus had been killed. The nucleus may remain uninjured, dividing normally; or it may become fragmented or vacuolated. When the nucleus remains uninjured, it divides normally even in greatly injured protoplasm. The maximum of injury to the protoplasm in which the nucleus can still divide cannot of course be determined from the sections. Karyokinesis is clearly seen to take place in the injured as well as in the uninjured cells, until the beginning of gastrulation. In cases of vacuolisation, the nuclei become large and clear and threads and dots are seen in them. Sometimes the chromatin is aggregated at the side of the nuclear membrane. No division of these nuclei has been discovered although all the nuclei in the cellulated portion may be vacuolated. In early developmental stages, the nuclei are surrounded by dense pigment, and the nuclei themselves are large and do not stain deeply. In blastula and gastrula stages less pigment is present around the nuclei, and the latter are small and stain deeply.

The protoplasm appears to be less resistent than the nucleus to heat and to mechanical injuries. The protoplasm may be displaced, and a streaming movement seems frequently to follow the operation with a hot needle. In some cases, the walls between the injured and uninjured blastomeres are broken down, bringing about a mingling of the protoplasm. From this it is obvious that nucleolisation of the injured from the uninjured blastomere might easily take place, if the nucleus of the injured blastomere were killed or its division delayed. In an egg so injured there is no evidence to show that the regular sequences of division follow; but sections do show that a small part of an egg, interiorly situated may become cellulated without giving any external evidence of the fact. Vacuolisation does not prevent the protoplasm from forming cells: on the contrary, greatly vacuolated protoplasm becomes cellulated and passes through the various developmental stages to the gastrula, beyond which it has not been observed. The protoplasm of the exovates is also frequently seen to become cellulated, the cell-division continuing for several days.

The yolk is easily injured and becomes disorganized as a result of comparatively slight injuries. Division of the protoplasm follows nuclear division in the majority of cases; but nuclear division in the injured yolk frequently takes place without a corresponding division of the yolk. The peripheral portion, richer in protoplasm divides first in case of injury, and then division proceeds towards the interior of the yolk.

In eggs killed immediately after the operation it is not possible to tell whether the nucleus of the operated blastomere has been killed. After an hour or more its condition can be more accurately ascertained.

Since it is practically impossible, with the method employed to injure all eggs in the same way or to the same extent; and since the subsequent history of an egg depends largely upon the extent of the injury, it is difficult to make a general statement of results. Therefore, in the following account, where a detailed description is given, the conditions in some individual eggs are taken as more or less typical of the conditions in all, although many different combinations of conditions will remain unnoticed. The blastomere which has been operated upon is spoken of as »injured«; the blastomere not operated upon as »uninjured«.

Eggs killed immediately after the operation or ten minutes later show one or more of the following conditions:

Egg no. 1. No visible effect except an outflow of substance, and a heavy deposit of pigment in the injured region.

Egg no. 2. The wall between the injured and uninjured blastomeres has become ruptured, and a mingling of the contents of the two blastomeres has followed.

Egg no. 3. The division-wall in this egg, stuck three-fourths of an hour after segmentation began, has disappeared. The nucleus of the uninjured blastomere is in karyokinetic division. The condition of the nucleus in the injured blastomere cannot be made out with certainty.

Egg no. 4. In the interior of the egg, in the region of the nucleus, there are rather large areas of granular material, the origin of which is not clear. The nucleus of the uninjured blastomere is apparently uninjured and in process of division. The condition of the nucleus in the injured blastomere could not be ascertained.

When killed one-half hour after the operation the conditions appear to be similar to those described in eggs killed immediately. In some cases, the nucleus, but not the protoplasm of the operated blastomeres has divided, while the uninjured blastomeres show normal division. The granular areas already spoken of are often conspicuous. Injury to and displacement of the wall between injured and uninjured blastomeres as seen in Fig. 1 is frequent and in one case, at least, the yolk-parts had become continuous.

After one hour, the egg may appear to be in the same condition as those killed immediately after the operation; or the uninjured blastomeres may have divided normally, the injured abnormally, according to the extent of the injury. In this series the following conditions were found in three eggs:

Egg no. 1 (Fig. 2). Two of four blastomeres were stuck. The division of the nucleus and of the protoplasm has proceeded normally, the division taking place regularly but more slowly in one of the two injured blastomeres. In this blastomere, the nucleus only has divided preparatory to the fourth division while in the uninjured blastomeres the protoplasm also has divided.

Egg no. 2. One of two blastomeres had been stuck. No nucleus found in the injured blastomere. Nuclear division had taken place in the uninjured blastomere.

Egg no. 3. One of two blastomeres had been stuck. The wall between the injured and the uninjured blastomere had been drawn toward the point of injury and the nucleus and protoplasm of the injured blastomere had been displaced.

After two hours, the protoplasm in the uninjured blastomeres had divided, the nuclei of the injured blastomeres had also divided, but the protoplasm showed peripherally, indentations only, not complete division.

In other eggs no changes seem to have taken placc that are different from those shown when the egg was killed at once. For this series, the following cases will indicate the results:

Egg no. 1. In this egg, the two anterior of the first four blastomeres had been stuck. In the two injured blastomeres, nuclear division had taken place, but the protoplasm gave no indications of division. One of the uninjured blastomeres had divided regularly and corresponded to the normal 16-cell stage; the second uninjured blastomere exhibited irregular indentations of the protoplasm only, but not complete division. The nuclei appeared normal and were surrounded by pigment. The cell-wall between the first two blastomeres could not be found.

Egg no. 2. One of two cells had been stuck. No division of the nuclei or protoplasm had taken place since the injury was made. The wall between the injured and the uninjured blastomeres had been ruptured in the middle third of the egg.

Egg no. 3. The wall between the two uninjured blastomeres has become greatly displaced, but is not broken. The wall between the two injured blastomeres has disappeared except in the middle of the egg where it is present. No nuclear division has taken place in any blastomere.

In sections of an egg killed after three hours, in which one or two blastomeres had been stuck, the uninjured blastomere has divided normally and the nucleus of the injured half has divided several times, but no division of the protoplasm has followed. In sections of an egg where two of four cells had been operated upon, the walls had been broken down effecting an interchange of material between the injured and uninjured blastomeres; nuclei in karyokinetic division were seen, but neither protoplasm nor yolk have divided.

An egg killed four hours after an operation on one of two blastomeres showed the uninjured blastomere in a stage corresponding to a normal one-half-16-cell stage; i. e., it contained eight cells with the planes of cleavage normal. The nucleus in the injured blastomere had been killed.

In four eggs, in which two of four blastomeres had been operated on, the yolk-part had not divided; in two of these eggs the entire protoplasmic part had divided; in the other two, the more protoplasmic part was nucleated but not cellulated.

After six hours, an egg in which one of two blastomeres had been stuck showed no indications of cell-walls, but several nuclear divisions had taken place. In another, the upper hemisphere had divided and appeared like that of a normal morula; no division of the yolk could be made out, although a few nuclei were seen in it. The middle of the yolk had become disintegrated.

In an egg killed after six hours, in which two of four blastomeres had been stuck, no cell-walls could be seen, but the nuclei were dividing. In another, the entire upper half of the uninjured blastomere had divided, and corresponded to a normal 16-cell stage, but the yolk contained no nuclei.

Fig. 3 represents a section through an egg in which two of four blastomeres were stuck. The demarcation line between one injured and one uninjured blastomere is very distinct, as seen in the figure. The uninjured blastomere has divided normally and delamination has begun. The nucleus of the injured blastomere has divided and the nuclei are surrounded by dense pigment deposits, but no cell-formation has followed. In sections through the other injured and uninjured blastomeres of the same egg (not figured) the more protoplasmic part of the injured blastomere has divided, the yolk is nucleated but undivided. This egg illustrates the complexity of the conditions following an injury to two of four blastomeres and shows how unsafe it is to draw conclusions from comparatively late stages of development only; for, in the blastula and gastrula stages, the boundaries between the blastomeres become less distinct, and it is impossible to determine absolutely the origin of certain cells the position of which leaves their origin in doubt.

After seven hours, when two of four blastomeres had been stuck, the uninjured blastomeres exhibited an irregular division corresponding to the normal morula-stage, while a few cells had formed in the injured ones, farthest from the point of injury.

After eight hours an egg in which one of the first two blastomeres had been stuck, shows a great number of nuclear divisions in the upper half of the egg, but the nuclei are vacuolated; granular areas appear in the yolk, and there are indications of cell-division in the lower hemisphere.

Between the eight and twenty-four hour series, no eggs were killed. In sections of embryos killed twenty-four hours after sticking one of the first two cells, the following results were obtained:

Egg no. 1. The walls between the injured and uninjured blastomeres seem to have disappeared, and the entire protoplasm and yolk of the two have become greatly vacuolated; nevertheless, both protoplasm and yolk are nucleated throughout, but no cells have formed in any part. The nuclei are surrounded by pigment.

Egg no. 2. The more protoplasmic part of both injured and uninjured blastomeres has divided, but a blastula cavity has not formed, and the yolk is not nucleated. The cells are round, and those in the interior are sometimes smaller than those at the surface. The nuclei are surrounded by pigment.

Egg no. 3. The more protoplasmic parts of both injured and uninjured blastomeres have become cellulated, and a portion of the yolk adjacent to the cellulated protoplasm has divided. No blastula-cavity has formed, and the cells are round or irregular in outline. The condition of the nuclei could not be ascertained.

Egg no. 4 (Fig. 4). One of two blastomeres had been stuck. The entire uninjured blastomere has divided. The protoplasmic portion of the injured blastomeres is nucleated; the yolk-part is sparcely nucleated. The uppermost portion is divided into cells continuous with those forming the roof of the segmentation-cavity. In the periphery of the injured blastomere and close to the point of injury, at a point farthest from the cellulated parts of the uninjured blastomere, a small group of cells is seen. Since this group of cells is removed from the uninjured blastomere and connected with the other cells by only a single line of superficial and imperfectly separated cells, there can be little doubt that the nuclei of these cells have been derived from the nucleus of the injured blastomere. Fig. 5 represents a section of the same egg cut at a different level. This shows the injured part nucleated but not cellulated.

In the following series, two of the first four blastomeres were injured, and the eggs killed after twenty-four hours:

Egg no. 1. The protoplasmic part of the two injured blastomeres has divided into large and irregular cells containing nuclei surrounded by dense pigment. The yolk-part of the injured blastomeres contains a few nuclei, but no cells have formed and a blastula-cavity is not present. The protoplasm of the uninjured blastomeres has also divided irregularly. A blastula cavity is present with the undivided but nucleated yolk as the floor.

Egg no. 2. The injured blastomeres contain a few large scattered nuclei surrounded by dense pigment. Whether cells have been added to the roof of the segmentation cavity from the injured blastomeres cannot be determined. The uninjured part is divided.

Egg no. 3. There is a clear demarcation line between the injured and the uninjured blastomeres. The entire protoplasm and yolk of one uninjured blastomere has divided, a well developed »half« segmentation cavity is found in the cellulated part. The protoplasm of the second uninjured blastomere has also divided, with the exception of a portion adjacent to one of the injured blastomeres in which no nuclei can be seen. The more protoplasmic part of the two injured blastomeres is nucleated and a few cells lying at the edge of the segmentation-cavity, have become separated off.

Egg no. 4. Injured and uninjured blastomeres have divided into about twenty cells. The nuclei appear normal and are surrounded by dense pigment.

Egg no. 5 (Fig. 6). The walls between the two injured and the two uninjured blastomeres are distinctly seen, and is unbroken throughout its extent. The uninjured blastomeres have become cellulated, the cell-walls being indistinct in the yolk-part. A blastula-cavity has formed and presents the appearance of a »half-blastula« cavity. The material of the injured blastomeres is nucleated, the nuclei being surrounded by dense pigment. A single cell has formed at the surface of the more protoplasmic portion of one injured blastomere, not far from the boundary between the injured and uninjured blastomeres. But since the first wall between the two blastomeres gives no evidence of having been broken, and since this cell is not immediately adjacent to the cells of the uninjured blastomere, it is probable that the cell has formed about a nucleus which originated from the nucleus of the injured blastomere. The exovate is not large and appears to contain no nuclear material.

In eggs killed forty-eight hours after one of two blastomeres has been operated upon, the following results were obtained:

Egg no. 1 (Fig. 7). In this egg the yolk of the uninjured blastomere is not completely divided, the more protoplasmic part, however, is divided into small cells. A large segmentation-cavity is present the roof of which extends over onto the injured side. No nuclei are present in the yolk of the injured side below the level of the injury which is somewhat to the side. Whether the cells of the roof of the segmentation-cavity on the injured side have come from the nucleus of the injured blastomere, or whether the cells of the uninjured side have extended over in this direction, cannot now be definitely determined.

Egg no. 2. The egg is filled with pigment-spots containing, without doubt, nuclei. The only cells present are, curiously enough, those in the region of the injury. The other half and much of the injured part is not cellulated.

When the two anterior of the first four blastomeres were stuck and the egg killed after forty-eight hours, the following conditions were seen:

Egg no. 1 (Fig. 8). Both of the uninjured blastomeres have divided, the boundary between the injured and the uninjured being clearly marked in the yolk-region below the blastula-cavity. In the roof of the cavity, the material of which has divided very atypically, the wall between the blastomeres seems to have disappeared and the material of the blastomeres has become continuous. The injured blastomeres are vacuolated but nucleated. It is not possible to ascertain definitely whether any cells have formed in the more protoplasmic part near the roof of the blastula-cavity. In the uninjured blastomeres an invagination has begun, which is probably the posterior lip of the blastopore.

Egg no. 2. The more protoplasmic parts of both injured and uninjured blastomeres have divided, and exhibit small, round cells, with apparently normal nuclei. The yolk of the injured blastomeres has become nucleated but not cellulated; the yolk of the uninjured blastomeres is cellulated, the nuclei normal, and an invagination, probably the posterior lip of the blastopore, has begun. The exovate exhibits cells which appear in form and size, like those of the cellulated protoplasmic parts in the blastomeres.

Egg no. 3. There is a clear demarcation line between the injured and uninjured blastomeres. The peripheral portion only of a part of the more protoplasmic region of the injured blastomeres has divided, and the yolk is greatly vacuolated and disorganized. Both protoplasm and yolk of the uninjured blastomeres have divided and the nuclei are small and deeply stained.

Egg no. 4 (Fig. 9). In this egg there is a large segmentation-cavity covered by a roof of small cells (three or four layers deep). The protoplasm and · the yolk of the uninjured side is divided into cells that appear to be normal. An indentation on the side, produced by the drawing in of yolk-cells whose outer ends are pigmented, probably represents a blastoporic rim (posterior lip?). The yolk of the two injured cells below the region of injury is entirely without nuclei. This means, either that nuclei of the two injured cells have been drawn out into the exovate (of which there is no direct evidence and which has not been shown in any other case) and the roof of the segmentation cavity has extended over to the other side, or, the upper part of the injured blastomeres have divided and helped to form the roof of the segmentation-cavity, but none of the nuclei have been able to pass through the injured region into the yolk below.

Egg no. 5. The two uninjured blastomeres have divided regularly and exhibit normal cells and nuclei. The peripheral protoplasm of the two injured blastomeres has divided normally, and, together with the two uninjured blastomeres, formed an abnormal gastrula having a very large yolk-plug. The invagination is deep on the uninjured side, and is pigmented, resembling more the posterior end of the gastrula. Only faint traces of the dorsal lip can be seen and even this is uncertain.

Egg no. 6. The protoplasmic parts of the injured and uninjured blastomeres have become greatly vacuolated. A superficial group of cells containing abnormally large nuclei has formed on the top of the egg. There are no other cells. The yolk has become nucleated throughout, but not cellulated. A blastula cavity is not present, but large vacuoles are found in the interior of the egg.

Egg no. 7. The more protoplasmic parts of the two uninjured blastomeres have divided into irregular cells, some of which contain abnormal nuclei, others apparently normal nuclei. The yolk is vacuolated but nucleated, and in the region adjacent to the cellulated, more protoplasmic parts, it is also cellulated. The protoplasm of the injured blastomeres is cellulated only at the surface and adjacent to the injury, because here the protoplasm has accumulated after the outflow following the injury. The yolk of the injured blastomeres has become nucleated, but no cells have formed.

The embryos killed after three and after five days were all from eggs in which the two anterior of the first four blastomeres had been stuck. A detailed description is unnecessary as the conditions are in many ways similar to those described for the forty-eight hour series, with the difference that some of the eggs had advanced further in development. Among the eggs killed after three days, some had not gone further than the blastula-stage, others had gastrulated. In the greater number, the protoplasm of the entire egg is divided, and the yolk is sometimes nucleated, sometimes non-nucleated, but usually, even when nucleated, not cellulated. In many such embryos, particularly in those which had gastrulated, the nuclei are small, round, and deeply stained, the cells small and regular.

Figs. 8, 10, 11, 12, represent transverse sections through the anterior, middle, and posterior regions of an embryo five days old.

From Fig. 10, which represents a section through the anterior end of the embryo, it is seen that in this region the nerve-plates are almost complete; i. e. the one on the uninjured side appears like that in a normal embryo, while the other is not fully developed. A mesoderm layer has formed on both sides, the one on the injured side being narrower than that on the uninjured side, but continuous with it. Ectoderm and mesoderm cells are small and contain normal nuclei; but some of the yolk-cells are atypical, and their nuclei are abnormally large or surrounded by dense pigment.

Fig. 11 is a section through the middle of the embryo. The nerve-plate of the injured side is greatly reduced in size. The chorda is seen to occupy a median position with respect to the nerve-plates, a position which it would occupy in the normal embryo. Some mesoderm is seen in the more protoplasmic, dorsal side of the injured part, but the cells in the yolk are irregular in form and size and do not form distinct germ-layers. A large area in the interior of the yolk is disorganized.

Fig. 12 shows a section through the region of the blastoporic invagination which extends over to the undivided material of the injured half, which is nucleated but not cellulated. From the preceding account it is evident that most of our results that are new relate to the earlier stages, for it appeared to us more important to determine what the immediate results of the operation really are, than to make a study of later stages without a sufficient knowledge of what effects the operation really bring about. We have found abundant evidence that in almost all cases the nucleus of the injured blastomere is not killed, and that it subsequently supplies the protoplasm of the injured blastomere with nuclei that become the center of cell-formation. We have found no evidence of a transmigration of nuclei from the uninjured to the injured side, and have not seen anything that would lead us to believe that the injured protoplasm becomes reorganized by nuclei or by cells wandering over from one side to the other as Roux claims takes place.

The development of the injured blastomeres appears always to be due to the nuclei of those blastomeres. The division may, it is true, be delayed, and in consequence the development of the injured half may lag behind that of the developing half. The sudden appearance of the missing half, described by Roux is probably due to the fact that the injured half has only been delayed in its development and has not been reorganized from the more active half.

Summary.

Our conclusions are in some respects similar to those of Roux; in other respects they are different.

Roux has admitted the possibility of so many processes taking place during reorganisation and postgeneration that it is not surprising to find that many of our results are in harmony with some of his conclusions. Our points of difference consist, therefore, in the main, in eliminating from the result a number of processes that Roux describes as taking place. It seems to us that much that is obscure in Roux's account of the processes of revivification, reorganisation and postgeneration is put in a much clearer light when we simply refer all such phenomena to the retarded development of the injured blastomere.

In making any comparison between our results and those of Roux it should not be forgotten, that, 1) we have worked on a different species of frog; 2) we have operated on eggs at the beginning of the laying period, instead of at the end as Roux recommends in order to obtain the best results in postgeneration; and 3) we have intentionally made the operation very severe in order to delay or prevent the development of the injured blastomere as far as possible. With this understanding the following points of resemblance and of difference come to light.

The embryos which Roux calls »half-embryos« appeared in our experiments when one of the first two blastomeres was stuck. We also obtained what appear to be posterior embryos when the two anterior of the first four blastomeres were stuck. Certain limitations should however be put on this statement. It is certain that a blastopore rim appears in the uninjured part, and that this does not appear to be a dorsal lip, but whether it would show the differentiation characteristic of the posterior lip can not be stated definitely, although such appears to be the case, and is best seen when this part becomes continuous with a retarded anterior lip in the injured part.

We have found that the nucleus of the injured blastomere usually continues to divide and supplies the injured protoplasm with nuclei. Around these as centers, cells are often produced which may take part in the formation of the embryo and make it more than »half«. Roux also found that this process takes place, although he lays much less emphasis on this side of the development than we should do. We have not studied in sufficient detail to make a comparison of any value the second and third methods modes of reorganisation described by Roux (viz., the »revivification« of parts of the protoplasm that have been greatly injured, and the growth of the ectoderm from the injured over the uninjured side). The principal point of difference between our results and those of Roux is one of some importance. We have found no evidence of the transmigration of cells or of nuclei from the uninjured to the injured side, and consequently we are inclined to believe that this process plays little or no part in the »reorganisation« (?), or better in the development, of the injured blastomere. Roux has written to one of us that he also is now inclined to lay much less stress on the evidence from this side of his work.

Hertwig repeated Roux experiment and reached conclusions that are in many ways diametrically opposed to those of Roux. It appears to us that the difference in the two cases are due largely to the incompleteness of the experiment performed by Hertwig, in so far as he failed to injure sufficiently the blastomere that was stuck with the needle. In consequence of this, the injured blastomere continued to divide and thus to contribute cells to the embryo. Owing to this, HERTWIG obtained unsatisfactory results from which we believe he has drawn several erroneous conclusions. His results may also be due in part to the rotation of the contents of the uninjured blastomere after the operation. HERTWIG's failure to obtain »half-embryos« is probably due to the former or to the latter conditions of his method, or to both combined. Later work on the frog, and on other forms as well, has abundently shown that »half-embryos« and even more incomplete parts may be produced.

HERTWIG's contention that the injured part of the egg assumes the rôle of the yolk-material in a meroblastic egg seems wide of the mark, as one of us has pointed out on several previous occasions. We do not think that there is anything in HERTWIG's own results, or in those of others, that gives a basis for such an interpretation. In fact this point of view appears to us to give an entire misconception of what the results of this experiment really are. ROUX's main conclusion in respect to the kind of embryo, »half-embryo«, that is first formed, is we believe correct, provided one blastomere fails to develop. HERTWIG's conclusion that half-embryos do not develop under these conditions is not correct.

On the other hand we agree with much that HERTWIG has said in respect to the so called »revivification« of the protoplasm which ROUX supposes to occur. The development of the injured blastomere appears to us to be due to its own nucleus dividing and supplying the protoplasm with centers around which the protoplasm forms cell-walls.

CURT ZIEGLER has recently given a very thorough and detailed treatment of the results of ROUX's experiment. Our results are in most essential respects in accordance with his; more particularly in that we find no evidence of transmigration of cells from the injured to the uninjured side, and find nothing that indicates a »revivification« of the injured protoplasm by means of wandering cells or nuclei from the uninjured part. If, as now appears to be the case, ROUX no longer wishes to press this point of transmigration of cells, then ZIEGLER's results are not so very different from those of ROUX, as one might gather from the elaborate criticism that he has made of ROUX's work. The most important outcome of the operation, namely the formation of a half embryo from the uninjured blastomere was also obtained by ZIEGLER, who confirms in this respect the earlier results of ROUX, ENDRES and WALTER, and MORGAN.

Our results differ from those of ZIEGLER in certain minor points.

We have paid more especially attention to the immediate effects of the operation and the changes that follow soon after. ZIEGLER's three grades of injury caused by the heated needle represent only in a very general way the principal changes that occur. In reality a much greater variety of results may follow than his classification might lead one to suppose. ZIEGLER admits the possibility of ROUX's third method of reorganization. Our own experiments are, as has been said, inconclusive on this point: — at least while they do not disprove that this may occur yet they show clearly in a number of cases that the superficial cells of the injured half are derived from the nucleus and protoplasm of that half, and have not migrated over from the uninjured part. We should like even to raise the question whether overgrowth of the injured half ever takes place to any extent unless some at least of the superficial cells originate from the injured half itself.

Zusammenfassung.

Unsre Schlüsse sind in einigen Beziehungen denen ROUXs ähnlich; in andern Hinsichten sind sie von ihnen aber verschieden.

ROUX hat die Möglichkeit des Eintretens so vieler Prozesse während der Reorganisation und der Postgeneration zugelassen, daß es nicht überraschen kann, wenn wir manche unsrer Ergebnisse in Übereinstimmung mit seinen Schlüssen stehen sehen. Unsre Differenzen bestehen demnach hauptsächlich in der Elimination einer Reihe von Prozessen aus dem Ergebnisse, deren Eintritt ROUX schildert. Es scheint uns, daß vieles, was in der ROUXschen Aufzählung der Wiederbelebungs-, Reorganisations- und Postgenerationsprozesse dunkel ist, in ein viel helleres Licht gestellt wird, wenn wir alle solche Erscheinungen einfach auf eine Entwicklungsverzögerung der verletzten Blastomere zurückführen.

Beim Vergleich unsrer Resultate mit denen ROUXs muß man nicht vergessen, daß wir 1) mit einer andern Froschart gearbeitet haben, 2) daß wir Eier vom Anfang der Laichperiode benutzten, anstatt vom Ende derselben, wie ROUX empfiehlt, um die besten Postgenerationsergebnisse zu bekommen, 3) haben wir absichtlich den Eingriff möglichst schwer gestaltet, um die Entwicklung der verletzten Blastomere, soweit nur möglich, zu verzögern oder zu verhindern. Bei richtigem Verständnis dieser ebenerwähnten Tatsachen werden die im folgenden erwähnten Ähnlichkeits- und Differenzpunkte ins richtige Licht gesetzt.

Die Embryonen, welche ROUX Hemiembryonen nennt, traten bei unsern Versuchen auf, wenn eine der zwei ersten Blastomeren angestochen wurde. Wir erhielten auch den hinteren Halbembryonen ähnliche Gebilde, wenn die beiden vorderen der vier ersten Blastomeren angestochen worden waren. Immerhin verlangt diese Feststellung eine gewisse Einschränkung. Sicherlich erscheint eine Blastoporusspalte, welche nicht der dorsalen Lippe zu entsprechen scheint, in dem unverletzten Teil, aber ob sie auch die für die hintere Lippe charakteristische Differenzierung zeigt, kann nicht abschließend festgestellt werden, —

wenn dies auch der Fall zu sein scheint, wie am besten zu erkennen, wenn sich dieser Teil mit einer im verletzten Teil verzögert gebildeten vorderen Lippe zu einem Continuum vereinigt. (Roux erhielt auch bloß Semigastrula posterior, keine Hemiembryo posterior. Ges. Abh. II. S. 446.)

Wir haben gefunden, daß der Kern der verletzten Blastomere gewöhnlich fortfährt, sich zu teilen, und das verletzte Protoplasma mit Kernen versorgt. Um diese als Zentren entstehen oft Zellen, welche sich an der Bildung des Embryo beteiligen und ihn zu mehr als einer »Halbbildung« machen können. Roux fand auch, daß ein derartiger Prozeß stattfindet, legt darauf aber viel weniger Nachdruck, als wir tun sollten. Wir haben die Reorganisationsweisen bei der zweiten und dritten Versuchsmethode, welche Roux beschreibt (nämlich die »Wiederbelebung« stark geschädigter Protoplasmateile und das Wachstum des Ectoderms von der verletzten auf die unverletzte Seite herüber), nicht mit genügenden Einzelheiten studiert, um daraus irgendwie bindende Schlüsse zu ziehen.

Der Hauptdifferenzpunkt zwischen unsern und Rouxs Ergebnissen hat eine gewisse Tragweite. Wir haben kein Hinüberwandern von Zellen oder Kernen von der unverletzten auf die verletzte Seite wirklich beobachten können und sind infolgedessen geneigt, diesem Prozeß gar keine oder eine nur geringe Rolle bei der Reorganisation (?), oder besser, bei der Entwicklung der verletzten Blastomere zuzugestehen. Roux hat einem von uns geschrieben, daß er jetzt gleichfalls weniger Wert auf die nach dieser Seite hin gezogene Folgerung aus den nur in zwei Fällen gemachten direkten Beobachtungen zu legen geneigt ist.

Hertwig wiederholte die Rouxschen Versuche und kam zu Schlüssen, welche in mancher Beziehung denen Rouxs diametral entgegengesetzt sind. Es scheint uns, daß der Unterschied zwischen den beiden Fällen in weiter Ausdehnung durch die Unvollständigkeit der von Hertwig angestellten Versuche verschuldet ist, insofern, als er die angestochene Blastomere genügend zu schädigen versäumte. Infolgedessen fuhr die verletzte Blastomere fort, Teilungserscheinungen zu zeigen und Zellen zum Embryo beizusteuern. Aus diesem Grunde erhielt Hertwig nur unbefriedigende Ergebnisse, aus denen er unsrer Meinung nach irrige Schlüsse zog. Seine Ergebnisse können auch teilweise durch die Drehung der unverletzten Blastomere nach der Operation veranlaßt sein. Hertwigs Unvermögen, »Hemiembryonen« zu erhalten, ist wahrscheinlich dem ersten oder dem letzteren Faktor seiner Methode zuzuschreiben, oder beiden zusammen. Spätere Arbeiten am Frosch und ebensogut an andern Tieren haben überreichlich gezeigt, daß »Hemiembryonen« und sogar noch unvollständigere Teilbildungen erzeugt werden können.

Hertwigs Behauptung, daß der verletzte Eiteil die Rolle des Dotters im meroblastischen Ei übernähme, scheint uns weit vom Ziel, wie der eine von uns bei mehreren früheren Gelegenheiten bereits betont hat. Weder in Hertwigs Ergebnissen, noch in denen andrer Autoren findet sich unsrer Meinung nach eine Stütze für eine solche Auffassung. Dieser Standpunkt scheint uns eine völlig falsche Auffassung der wirklichen Bedeutung solcher Versuche zu ergeben. Rouxs Hauptfolgerung bezüglich der fraglichen Art von Embryonen, der »Hemiembryonen«, die zuerst gebildet wurden, ist unsrer Meinung nach korrekt, vorausgesetzt, daß eine Blastomere sich zu entwickeln unterläßt. Hertwigs Schluß, daß sich unter solchen Bedingungen keine »Hemiembryonen« entwickeln, ist nicht richtig.

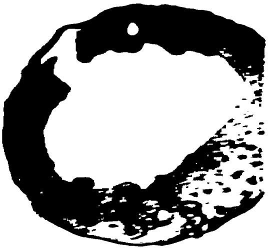
Anderseits stimmen wir mit vielem überein, was Hertwig bezüglich der sogenannten »Wiederbelebung« des Protoplasmas gesagt hat, deren Eintritt Roux voraussetzt. Die Entwicklung der verletzten Blastomere scheint uns durch die Teilung ihres eignen Kerns veranlaßt, durch ihre Versorgung mit Zentren, um welche das Protoplasma Zellwände bildet.

Curt Ziegler hat kürzlich eine erschöpfende und detaillierte Behandlung der Rouxschen Versuchsergebnisse veröffentlicht. Unsre Ergebnisse stimmen in den hauptsächlichsten Punkten mit den seinigen überein, speziell auch darin, daß wir keine Überwanderung von Zellen von der verletzten zur unverletzten Seite konstatieren können, und daß wir nichts finden, was für eine »Wiederbelebung« des geschädigten Protoplasmas auf dem Wege einer Überwanderung von Zellen oder Kernen aus dem unverletzten Teile spräche. Falls wirklich, wie es ja jetzt der Fall zu sein scheint, Roux nicht länger diesen Punkt der Zellüberwanderung ausdrücklich zu betonen beabsichtigt, dann sind auch Zieglers Ergebnisse gar nicht so sehr von denjenigen Rouxs verschieden, wie man aus seiner detaillierten Kritik der Rouxschen Arbeit schließen könnte. Das wichtigste Operationsergebnis, nämlich die Bildung eines Halbembryo seitens der unverletzten Blastomere, erhielt auch Ziegler, der diesbezüglich die früheren Ergebnisse von Roux, Endres und Walter und Morgan bestätigt.

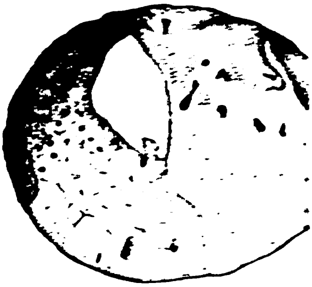
Unsre Ergebnisse unterscheiden sich von denjenigen Zieglers in gewissen, weniger wichtigen Punkten. Wir haben unsre Aufmerksamkeit ganz speziell auf die unmittelbaren Effekte der Operation und die bald danach folgenden Veränderungen gerichtet. Zieglers drei Grade der durch die heiße Nadel gesetzten Schädigung repräsentieren nur in sehr allgemeiner Weise die hauptsächlichsten auftretenden Veränderungen. In Wirklichkeit kann eine viel größere Verschiedenheit der Ergebnisse folgen, als diese Klassifikation einen zu glauben verleiten könnte.

Ziegler gibt die Möglichkeit der dritten Rouxschen Reorganisationsweise zu. Unsre eignen Versuche sind, wie gesagt, für die Beurteilung dieses Punktes unzulänglich: — jedenfalls, wenn sie auch nicht den Gegenbeweis gegen die Möglichkeit solchen Vorkommens liefern, so zeigen sie doch in einer Anzahl von Fällen deutlich, daß die oberflächlichen Zellen der verletzten Hälfte vom Kern und Protoplasma dieser selben Hälfte abstammen und nicht vom unverletzten Teil her eingewandert sind. Wir hätten sogar Lust, die Frage aufzuwerfen, ob ein Überwachsenwerden der verletzten Hälfte überhaupt in einiger Ausdehnung vorkommt, ohne daß wenigstens einige von den oberflächlichen Zellen von der verletzten Hälfte selbst abstammen.

1



4



6

10

LLS IN
EGG.

stages of
from the
cleavage
nterior of
arranged

ised by a
the egg
riod.
ipted to
ether the

ell stages
cleavage
any two
ime, the
ir on the

ervations
sions. I
between
regular
from the
ark pole
This is

vo
Te
we

de
de
da
Se
be
vo
wi
Ze
LE
au
wi
de
Er

we
die
än
Sc
au
de
leit

zu.
un
Mö
vo
Ke
letz
we
Au
Zel

Reprinted from BIOLOGICAL BULLETIN, Vol. VIII., No. 3, February, 1905.]

THE FORMATION OF THE INTERIOR CELLS IN THE SEGMENTATION OF THE FROG'S EGG.

MARGARET REED.

Sections through the egg of the frog in the early stages of segmentation show that the cleavage planes extend from the surface inwards forming but one layer of cells. Later cleavage stages, however, show certain cells entirely within the interior of the egg, so that the cells of the dark pole appear to be arranged in two layers about the segmentation cavity.

Former observers supposed this appearance to be caused by a cleavage plane coming in parallel to the surface of the egg (delamination) probably between the 32- and 64-cell period.

Following Professor Morgan's suggestion, I attempted to determine whether a delamination really occurs or whether the interior cells are produced in some other way.

If the delamination occurs between the 32- and 64-cell stages we should expect to find the interval between these cleavage stages, as seen on the surface, longer than that between any two preceding divisions, because somewhere during this time, the cells would have divided by planes, which do not appear on the surface.

In order to test this point, I made a number of observations on the living egg, noticing the times of the surface divisions. I found no marked difference in the length of the interval between any two divisions, but the cleavage took place in a regular rhythm. There was usually a period of about one hour from the first appearance of segmentation in the cells of the dark pole until the next cleavage again began to appear there. This is shown in the following table.

9:25 A. M.	Beginning of	2-cell	stage at	dark pole.
10:15 "	"	4	"	"
11:15 "	"	8	"	"
12:10 P. M.	"	16	"	"
12:55 "	"	32	"	"

1:50 P. M.	Beginning of	64-	cell stage	at dark pole.
2:40 "	"	128	"	"
9:25 A. M.		8	"	"
9:30 "	"	16	"	"
10:35 "	"	32	"	"
11:35 "	"	64	"	"
12:25 P. M.	"	128	"	"
1:40 "	"	256	"	"

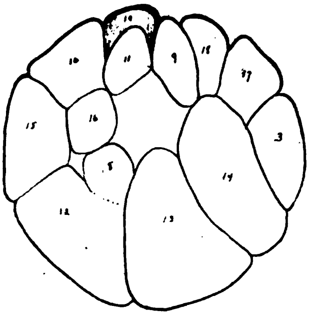
From this we see that if the delamination should occur between the 32- and 64-cell periods the division must take place at this period twice as fast as any other division. This of itself seemed improbable. I therefore preserved series of eggs taken every five minutes during the interval between the 32- and 128-cell divisions and later sectioned them in order to determine how the interior cells arise.

The eggs were cut about 20 μ thick and all the sections drawn with the aid of a camera lucida. Each cell of the egg was numbered and traced through the series of sections, in order to determine not only how many cells were present but also to ascertain how many came to the surface and how many were entirely inside. Two series taken every five minutes between the 32- and 128-cell stages were counted with the following as some of the results:

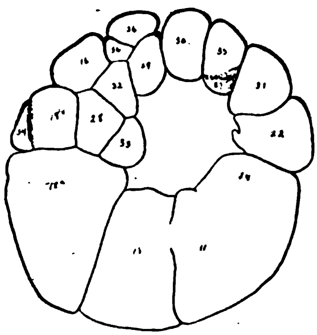
Supposed Number of Cells.	Actual Number of Cells.	Cells Inside.	Superficial Cells.
32	28	0	28
32	32	0	32
32–64	37	3	34
32–64	45	5	40
32–64	53	6	47
64	64	15	49
64–128	105	26	79

If delamination took place between the 32- and the 64-cell period, sections of an egg at the 64-cell stage should show 64 cells coming to the surface, plus a certain number of cells inside, but this, as the table shows, is not the case.

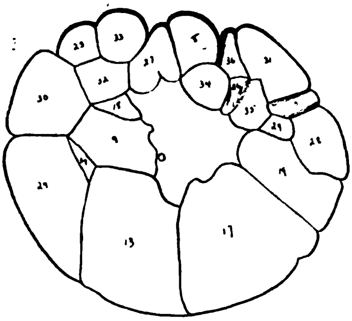
Sections through the egg as late as the end of the 32-cell stage show that all the cells are divided by cleavage-planes, which appear on the surface. Sections through the eggs of the series between the 32- and 64-cell stage show an increase in the number of superficial cells[1] as well as of those which are wholly within the egg. At the end of the 64-cell stage there are 64 cells present in the egg, a number, about 12, of which do not appear on the surface. Also the series between the 64- and 128-cell cleavage stages show an increase in the number of cells coming to the surface, as well as the number of cells of the interior, so that there is no cleavage stage between which and the following stage delamination takes place.



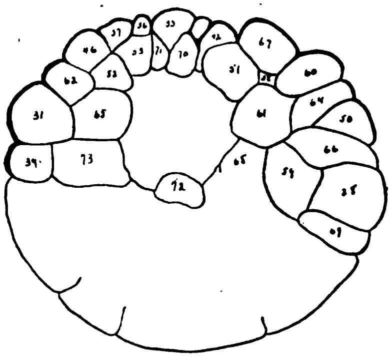
I



II



III



IV

Figs. 1, 2, 3 and 4 show sections through the middle of eggs of 28, 53, 64 and 105 cells respectively. A number of cells in these figures have the appearance of being pushed or crowded into the interior, that is, the portion of the cell which comes to the surface is quite small compared with the size of the cell which lies mainly within the egg.

---

[1] In the cleavage of the frog's egg, the division for the next cleavage stage begins in the cells of the dark pole, while the cells of the light pole are completing the division for the preceeding cleavage stage.

This appearance of the cells seems to be caused by the division planes coming in more or less obliquely so as to cut off one cell with a small surface area as No. 9, Fig. 1. At the next cleavage, the division of this cell would give rise to one cell entirely within the egg and one coming to the surface. Cell 27, Fig. 3, shows the beginning of such a division while cell 70 or 71, Fig. 10, may have been formed in this manner. The following division of such a cell would then form cells lying entirely within the egg.

So far as I have been able to trace, the yolk cells also show no cutting off of interior cells during the early cleavage stages. Such a cell as 72 in Fig. 4, is but the end of a cell which other sections show coming to the surface.

These observations show that there is no delamination division cutting off a number of cells parallel to the surface but that the first formed cells of the interior are produced by the same division planes as are the cells of the surface and by their subsequent division form the cells of the interior.

Columbia University,
December 13, 1904.

Nachdruck verboten.
Übersetzungsrecht vorbehalten.

Further Studies on the Ovogenesis of Sagitta.

By

N. M. Stevens.

(From the Biological Laboratory of Bryn Mawr College.)

With plate 16.

After the results of my work with Prof. Boveri on *Sagitta* (1903) were sent to the publisher, an attempt was made to obtain the material necessary to complete the study of the ovogenesis. Prof. F. M. MacFarland kindly offered to secure a new supply of *Sagitta* at Naples, and prepare it as was suggested by results already obtained. Only one specimen, however, threw any light on the doubtful points.

In April of the present year, I obtained from Mr. Geo. M. Gray, Curator of the Marine Biological Laboratory, Woods Hole, Mass., several dozen of *Sagitta elegans*, which gave me the desired stages, — in fact, all stages in the ovogenesis up to the actual extrusion of the eggs from the oviduct.

The one favorable specimen of *Sagitta bipunctata* from Naples contained six eggs which showed the first maturation spindle in equatorial stage; the eggs were in what appeared to be a temporary oviduct, formed between the sperm-duct and the germinal portion of the ovary (Pl. 16, Fig. 1). This rather surprising arrangement by which the germinal epithelium and the accessory cells of the larger oocytes are temporarily cut off from their usual connection with the sperm-duct, needed confirmation. This specimen, moreover, gave no hint of the way in which the ripe ova pass from the ovary proper into this temporary oviduct.

The new material from Woods Hole, *Sagitta elegans*, gave all stages in the formation of the oviduct and the entrance of the eggs; also the breaking down of the germinal vesicle and the formation of the first polar spindle, which occur before the egg separates from the ovarian wall.

Ripening of the ovum. — The conditions shown in tab. 20, figs. 2b and 16 of my previous paper (1903) were the latest stages found in that material. Here the chromosomes are fully reduced in size (fig. 2b), and the spermatozoon has entered the second accessory cell (fig. 16).

The nucleus now moves toward the periphery of the ovum until it comes into contact with the egg-membrane at a point opposite to the point of attachment of the ovum to the wall of the ovary. The small chromosomes, which up to this time have been scattered through the nucleus, are drawn into currents in the karyoplasm, and brought together at a point, usually near one end of the nucleus as it appears in cross-section. In the nucleus shown in Fig. 2, currents have appeared near the periphery and some of the chromosomes are already in the path of the currents. In Fig. 3a the currents flow toward a region where several of the chromosomes are grouped together, and two others are in the path of one of the currents (Fig. 3b). In Fig. 4 the spindle fibres are forming among the assembled chromosomes; the nuclear membrane on the side of the nucleus nearest to the spindle has disappeared and the karyoplasm appears to be flowing out into the cytoplasm. Fig. 5—8 show successive stages in the dissolution of the nucleus and the mingling of karyoplasm with cytoplasm. In Fig. 7 and 8 may be seen the peculiar disposition of the chromosomes in the equatorial plate of the spindle: each chromosome lies in a vesicle by itself and at this stage the spindle fibres are certainly not attached to the chromosomes. The spindle is barrel-shaped — broad and short — with no mantle-fibres and no centrosomes. At this stage, the spindle has a tangential position (Fig. 6—8), the radial position being assumed after the eggs have entered the oviduct (Fig. 1).

The separation of the egg from the wall of the ovary occurs as follows: The egg-membrane closes the micropyle, excluding the second accessory cell (Fig. 9). At this time both accessory cells show signs of degeneration and separation takes place between the accessory cells and the wall of the ovary (Fig. 9), or sometimes between the two cells (Fig. 10a and b), or between the second accessory cell and the egg. The epithelial cells on either side stain more deeply and appear to be denser than usual (Fig. 9 and 10a). The egg now pushes its may between the ovary and the sperm-duct, crowding the latter against the body-wall (Fig. 11). The epithelium of the ovary peels off from the wall of the sperm-duct leaving such smooth surfaces (Fig. 1, 11, 12) that one can hardly believe that the duct in which the ripe eggs lie is a temporary one, and that a short time before it was formed, sperm tubes had extended from the sperm-duct through the accessory cells to the oocytes; but there is abundant proof that this is true. In many cases eggs are found at intervals either passing from the ovary into the oviduct or lying wholly in it, while between such eggs are portions of the ovary which show no sign of the oviduct. The opening from the ovary to the oviduct seems to close immediately behind the egg, and in most cases the eggs certainly make their way into the oviduct independently, and the opening bears a direct relation to the point where the egg breaks away. A few eggs were found lying free in the ovary with no opening formed, and it is possible that they would follow eggs with which they were in contact into the oviduct through openings already made by the latter. The appearance of the eggs in the sections indicates that each individual egg makes its way into the oviduct by means of its own activity, but to be sure of this point one must observe this phenomenon in the living animal.

The number of eggs ripening in the two ovaries for one laying varies greatly. In the specimen of *Sagitta bipunctata* examined, there were three in each oviduct; in *Sagitta elegans* there were from 2 to 9 and as great a difference between the two ovaries of one individual as 4 and 8.

The Chromosomes. — In a few specimens of *Sagitta bipunctata*, the chromosomes in the stage shown in fig. 13e and 15 of tab. 20 (1903) were split longitudinally, but the preservation of the material was poor and it was feared that the appearance was not normal. In *Sagitta elegans*, however, I find this splitting to be a normal condition at a certain stage of the reduction in the size of the chromosomes. Two such chromosomes are shown in Fig. 13.

The two strands are always more or less twisted, but the ends are occasionally free. Large granules are usually seen in connection with these chromosomes.

The chromosomes in the equatorial plate of the first polar spindle (BOVERI 1890 and fig. 1, 6, 7, 8, 12) are split twice longitudinally forming a tetrad similar to that of *Ascaris*, but differing less in appearance from the ordinary chromosome, as the components of the tetrad lie very close together and are very long in proportion to their diameter. Comparison of a polar and a lateral view of the spindle reveals the fact that the split which shows in a polar view — that which corresponds to the second maturation division — is somewhat wider than that seen in a lateral view. An end view of a tetrad also shows a difference in the thickness of the components of the tetrads in the two diameters, as seen in Fig. 14 a. These facts as to the composition of the tetrad incline one to believe that we have here a bivalent chromosome with the two components paired longitudinally instead of end to end. If this is so, the first division divides both of the original chromosomes longitudinally, and the second division separates the chromosomes forming the pairs. The split observed in an earlier stage (Fig. 13) is probably a temporary separation of the longitudinally paired chromosomes.

In nine out of twelve ripe eggs of one individual, there were two polar spindles. These spindles were in various positions with respect to one another and to the egg-membrane, — 1) end to end and tangential, 2) parallel and radial (Fig. 15), 3) end to end and radial (Fig. 16). The two spindles seemed in most cases to be exactly alike. In one egg they were so close together as to form one very broad spindle, and in another the poles of the two spindles were united at one end but separate at the other (Fig. 17). The chromosomes, however, showed only one longitudinal cleft, indicating that the division of the first polar spindle had been completed, though this usually occurs only after the eggs are laid. One of these spindles is evidently the 2d polar spindle (Fig. 16 a) and the other corresponds to the division of the first polar body which has not been separated from the egg (Fig. 16 b). An end view of a dyad taken from one of these spindles in equatorial stage is shown in Fig. 14 b. In two eggs the spindles were in a late anaphase (Fig. 17), and the chromosomes were evidently the separated components of the dyads.

The only explanation of this abnormality seems to be that laying was prevented by some unusual conditions, and that the usual order of events so far as the distribution of the chromosomes is concerned went on, though for some unknown reason the polar body was not given off. No other peculiarity was observed.

The question now arises whether the sections throw any light on the method of reduction in number of chromosomes in the young oocytes. This is one of the questions that I had not been able to answer in my earlier work, but *Sagitta elegans* proved to be more favorable material than *Sagitta bipunctata*. Among the young oocytes are some which contain the somatic number of chromosomes (18), some V-shaped (Fig. 18), others shortened and irregular in form (Fig. 19). A few of these showed a part of the chromosomes arranged in pairs (Fig. 20). A somewhat later stage shows the reduced number of chromosomes (9) elongated, and some have a longitudinal split (Fig. 21 and 22), which evidently corresponds to the space between two chromosomes of a pair in the earlier stage, and probably to the longitudinal division shown in Fig. 13 and to the wider split in the tetrad of the first polar spindle (Fig. 14 a). In Fig. 23 is shown an oocyte in which such pairs of chromosomes as appear in Fig. 21 are seen in cross-section.

In the spermatogenesis of *Sagitta* we have the reduction effected by pairing of the chromosomes end to end, and the first maturation division separates the two chromosomes, while the second probably completes the longitudinal division that has been observed during the growth stage of the spermatocytes, between the two stages shown in fig. 28 a and b, tab. 21 (1903) (Fig. 24 a and b).

In the ovogenesis the method of pairing is different and the order of the maturation divisions is reversed, the second division separating the components of the pairs. From this it would appear that it is the "conjugation" of the chromosomes and their longitudinal division and separation that are important, and not the method of union or the order of divisions. There is need, it seems to me, of going over as carefully as possible the ovogenesis of all forms which have been described as undergoing, during the process of maturation, a double longitudinal instead of a longitudinal and a transverse division, for evidence whether or not the two kinds of divisions are equivalent.

Since in *Sagitta* we find two methods of "conjugation" of the chromosomes,—pairing end to end in the spermatocyte and longitudinal pairing in the oocyte; and the corresponding two types of maturation-divisions, — a transverse and a longitudinal in the spermatocyte, and two longitudinal divisions in the oocyte, giving, however, equivalent results in the nuclei of the egg and sperm; i. e., $\frac{a|a}{b|b}$ in the sperm and $\frac{a|b}{a|b}$ in the egg and its polar bodies, it seems probable that further investigation will show that in many if not in all cases the two theoretically conflicting types of maturation divisions are equivalent, the paired chromosomes being in all cases separated by one or the other of the maturation divisions. This question is of especial interest in connection with recent investigation of problems relating to Mendel's Law of Inheritance.

Brauer's work on *Ascaris* (1893) in connection with that of Boveri (1899) and others seems to indicate that the chromatin granules which Brauer describes as uniting in pairs before the spireme is formed are the true chromosomes; and if so, one of the longitudinal divisions of the tetrad of *Ascaris* really separates paired chromosomes.

Chromatin granules. — The question as to whether the granules thrown off by the chromosomes and cast out into the cytoplasm, pass from the nucleus to the cytoplasm in the form in which they are seen on both sides of the nuclear membrane (p. 230—231, 1903) is satisfactorily answered by the sections of *Sagitta elegans*. Fig. 25 shows these granules scattered through the nucleus, a few of them in contact with the nuclear membrane — some within, some without — and others in strands of karyoplasm which extend through the nuclear membrane out into the cytoplasm. This and many other similar sections show that the granules actually pass out of the nucleus along strands of karyoplasm. The nuclear membrane is not so definite a structure in the ripening oocytes of *Sagitta* as in many forms, though in earlier stages it is quite thick and is fortified on the outside by a deeply-staining reticulum which gradually disappears as the oocyte and the nucleus increase in size.

With the material at hand, I am unable to give any very definite opinion as to the composition of these granules which are the product of the gradual reduction in the size of the chromosomes. They have been spoken of as chromatin granules, because of their origin, and because they stain like the chromosomes in iron-haematoxylin preparations and hold the stain while much larger yolk granules are destained. The slide from which Fig. 25 was taken was stained and restained with several different combinations but not with very satisfactory results. With safranin and lichtgrün, the granules held the safranin, while the yolk granules took the green. This evidence is in favor of their chromatin nature, but is of course insufficient.

It is my purpose to secure more of this material another year for further study 1) of these granules, 2) of the embryology of the ovary and its ducts, and 3) of some points in the spermatogenesis.

Summary.

1. The so-called sperm-oviduct of *Sagitta* is merely a sperm-duct.
2. A temporary oviduct is formed for each laying, between the sperm-duct and the germinal epithelium. This duct appears to be opened up by the activity of the individual eggs pushing their way out of the ovary proper and in between the germinal epithelium and the sperm-duct.
3. The chromosomes preserve their individuality from the time when reduction in number occurs in the very young oocytes to their appearance as tetrads in the first polar spindle.
4. The scattered chromosomes of the germinal vesicle are collected by currents in the karyoplasm, at the point where the first polar spindle is formed.
5. There is a preliminary longitudinal splitting of the chromosomes during their reduction in size.
6. The tetrad is bilaterally symmetrical, indicating that it is composed of two chromosomes paired longitudinally.
7. The chromosomes in the young oocytes conjugate longitudinally, instead of end to end as in the spermatocytes.
8. In *Sagitta*, we have two types of conjugation of the chromosomes in oocyte and spermatocyte, and two corresponding types of maturation divisions, giving, however, equivalent results.
9. The chromatin(?) granules which result from the reduction in size of the chromosomes pass directly out from the nucleus into the cytoplasm along strands of karyoplasm.

Hopkins Laboratory, Pacific Grove, California,
June 21, 1904.

Literature.

BOVERI, TH., 1890, Zellenstudien, Heft 3, in: Jena. Z. Naturw., V. 24.

—, 1899, Ueber die Entwicklung von Ascaris meg., in: Festschrift C. VON KUPFFER, Jena.

BRAUER, A., 1893, Zur Kenntnis der Spermatogenese von Ascaris megalocephala, in: Arch. mikrosk. Anat., V. 42.

STEVENS, N. M., 1903, On the ovogenesis and spermatogenesis of Sagitta bipunctata, in: Zool. Jahrb., V. 18, Anat.

Explanation of figures.

Plate 16.

Fig. 1. Cross-section of an ovary of *Sagitta bipunctata*, showing ripe egg (*b*) in the temporary oviduct. *a* sperm-duct; *c* 1st polar spindle; *d* oocyte. ZEISS' obj. C, oc. 4.

Fig. 2. Section of germinal vesicle, showing currents and small chromosomes. ZEISS' obj. C, oc. 6.

Figs. 3a and 3b. Two sections of the same germinal vesicle, showing most of the chromosomes brought together by the currents. ZEISS' obj. C, oc. 6.

Fig. 4. Germinal vesicle breaking down at the end where the spindle is forming. ZEISS' obj. C, oc. 6.

Figs. 5—8. Successive stages in the dissolution of the germinal vesicle and formation of the 1st polar spindle. Fig. 5, ZEISS' obj. C, oc. 6; Fig. 6, ZEISS' obj. D, oc. 6; Figs. 7—8, ZEISS' obj. 1.5, oc. 4.

Fig. 9. Section showing sperm-duct (*c*), a part of a ripe egg (*e*) with degenerating accessory cells (*b*) on the egg-membrane which has closed under them, and the opening (*a*) left by the breaking away of the accessory cells. ZEISS' obj. D, oc. 4.

Figs. 10a and b. Similar but slightly earlier stage than Fig. 9. *c* and *d* degenerating accessory cells. ZEISS' obj. D, oc. 4.

Fig. 11. Cross-section of *Sagitta elegans*, showing one ripe egg just entering the oviduct (*a*), and another wholly in (*b*). Egg *b* shows two polar spindles end to end. *c* sperm-duct. BAUSCH & LOMB obj. 1 in. oc. C.

Fig. 12. Cross-section of an ovary of *Sagitta elegans*, showing one egg (*a*) in the oviduct and another (*b*) entering. Both eggs contain the first polar spindle in equatorial-plate stage and tangential position. ZEISS' obj. AA, oc. 6.

Fig. 13. Two chromosomes showing the preliminary longitudinal split which occurs during the growth stage of the oocyte. ZEISS' obj. 1.5, oc. 6.

Fig. 14a. End view of a tetrad. Zeiss' obj. 1.5, oc. 6.

Fig. 14b. End view of a dyad. Zeiss' obj. 1.5, oc. 6.

Fig. 15. Two polar spindles from eggs of the same individual as Fig. 11, showing dyads. Zeiss' obj. 1.5, oc. 6.

Fig. 16. Two polar spindles, end to end and radial, showing dyads. *a* 2d polar spindle; *b* division corresponding to that of the 1st polar body. Zeiss' obj. 1.5, oc. 6.

Fig. 17. Two polar spindles with inner poles united; a part of the chromosomes in another section. The chromosomes here are univalent. Zeiss' obj. 1.5, oc. 6.

Figs. 18 and 19. Young oocytes containing the somatic number of chromosomes (18). Zeiss' obj. 1.5, oc. 6.

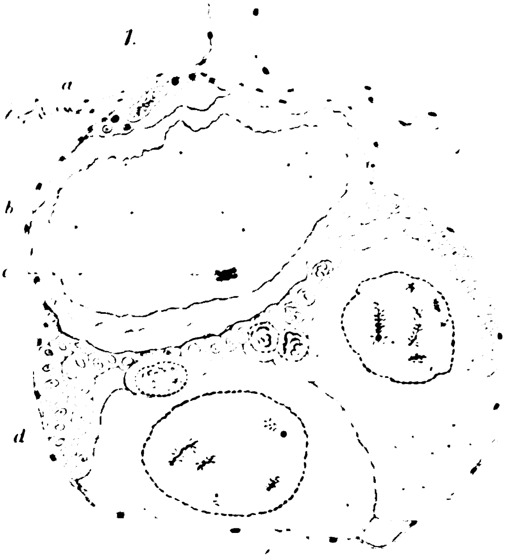
Fig. 20. Young oocyte showing paired chromosomes. Zeiss' obj. 1.5, oc. 6.

Figs. 21—23. Young oocytes showing longitudinally paired chromosomes. Zeiss' obj. 1.5, oc. 6.

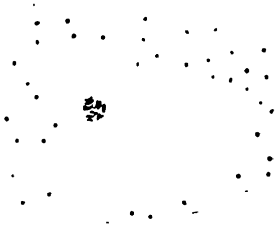
Figs. 24a and b. Spermatocytes showing preliminary longitudinal split. Zeiss' 2 mm, oc. 8.

Fig. 25. Section of nucleus of nearly ripe oocyte, showing chromosomes much reduced in size, and granules passing out along strands of karyoplasm. Zeiss' obj. C, oc. 6.

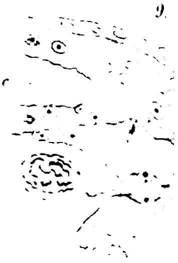
1.



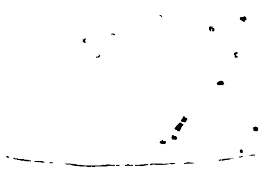
5.



9.

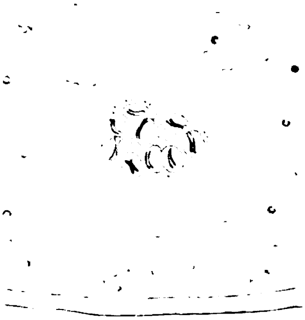


6.



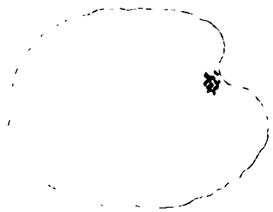
10a.

7.

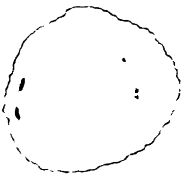


10b.

3a.

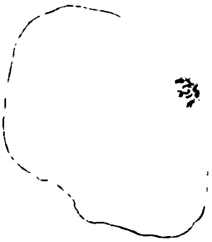


2.

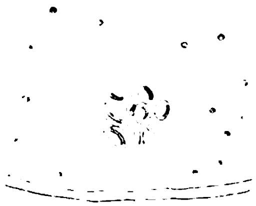


3b.

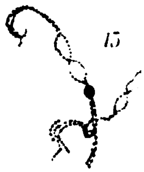
4.



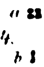
8.



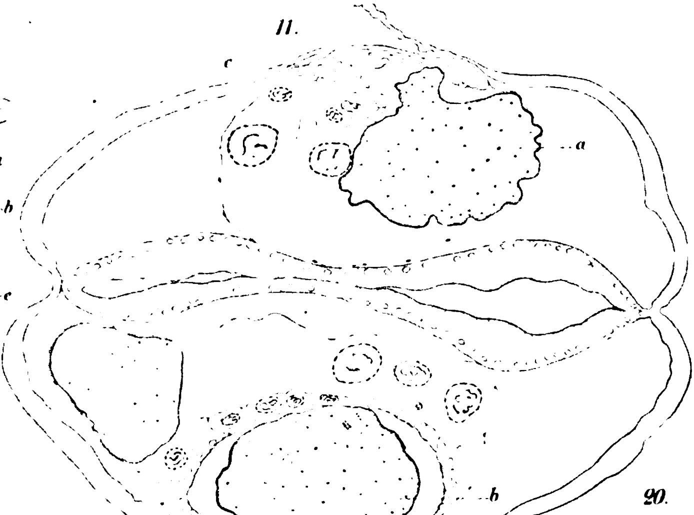
15.



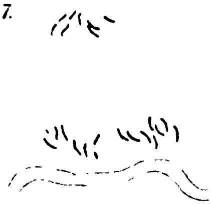
14.



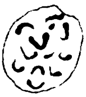
11.



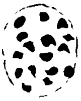
17.



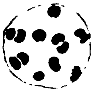
18.



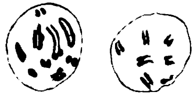
19.



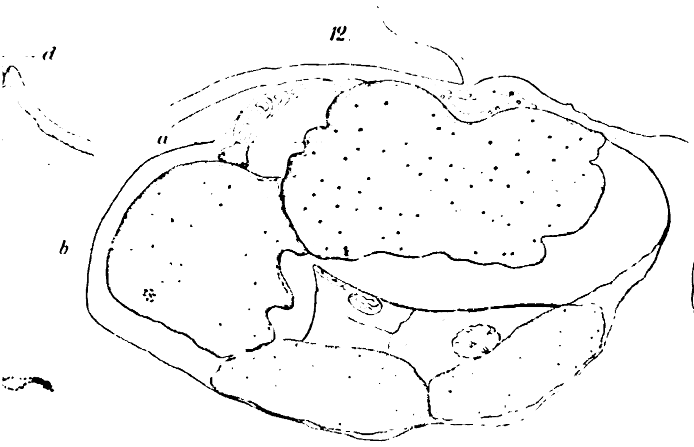
20.



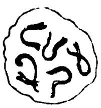
21.



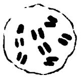
12.



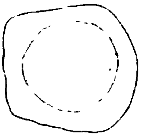
22.



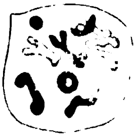
23.



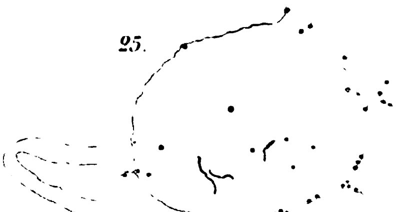
24 a.



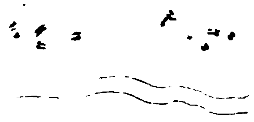
24 b.



25.



15.



16.

Fischer

EXPERIMENTS ON POLARITY IN TUBULARIA.

BY

T. H. MORGAN AND N. M. STEVENS.

WITH 5 FIGURES AND 20 TABLES.

In the discussion that has arisen in regard to the factors that determine the formation of new hydranths in *Tubularia*, the red pigment in the stem and in the current has played a conspicuous rôle. Loeb first suggested the idea in 1891 without, however, stating clearly whether he referred to the pigment in the wall or in the current, although the former appears to have been intended, as he makes no mention of a current or of pigment in the current. In his last publication, however, he argues in favor of the pigment in the current being an important factor in the regeneration of hydranths. Driesch also thought that the pigment in the circulation might have at least a quantitative relation to hydranth formation. Morgan has presented evidence which, he believes, disproves that the red pigment, as such, takes any part in the formation of the hydranth, pointing out, among other objections to the views of Loeb and of Driesch, that the pigment of the current is not absorbed, but is gathered together in the digestive cavity within the new hydranth; and Stevens has observed that this ball of pigment is later vomited forth from the mouth of the fully formed hydranth. We had hoped that our observations had laid to rest the question of the supposed rôle of the pigment in regeneration, but such appears not to be the case, since in his recent publication Loeb returns once more to the problem. For this reason it has seemed to us worth while to undertake a new series of experiments, although we think the evidence already advanced more than sufficient to disprove the view that the pigment has any direct influence on the regeneration of hydranths. While we have considered this question somewhat fully for the reasons given above, we think, however, that our new work is of more interest in connection with the problems of polarity and heteromorphosis in *Tubularia.*

The work was carried out during the summer of 1904, while enjoying the hospitality of the Hopkins Marine Laboratory of Leland Stanford Junior University. The species of *Tubularia* that is found near Pacific Grove is *T. marina.*

It differs in some respects from the forms found at Woods Hole and at Naples, the principal difference being that it has a single hydranth at the free end of an unbranched stem attached by stolons to the rocks. In regard to its powers of regeneration, it appears to be very similar to the other two forms, the main difference being its stronger tendency to produce a stolon from an aboral cut end rather than a heteromorphic hydranth.

GENERAL ARGUMENTS THAT ARE OPPOSED TO THE SUPPOSED FUNCTION OF THE RED PIGMENT AS A FORMATIVE OR NUTRITIVE FACTOR.

The discussion of the problem that follows may be made clearer, if we review here the points that bear on the supposed function of the red pigment as a formative or nutritive factor.

First. The stem of *Tubularia marina* contains throughout its length two ridges standing opposite to each other and nearly meeting in the middle of the stem. These ridges are connected by strands of endoderm at more or less irregular intervals, forming a perforated partition between the two circulatory canals of the stalk. The fluid in the central cavity of the stem moves up one canal, across to the other side of the partition just below the stomach of the polyp, and down the other canal. Long lash-like flagella, belonging to the endoderm cells, keep the fluid in circulation. Occasionally granules or food particles are contained in the fluid, but ordinarily it is quite clear and its movement is consequently difficult to demonstrate.

When a piece of the stem is cut off, its ends close rapidly, and the circulation continues as before. At first the fluid contains no granules, or only an occasional one. The first changes, preparatory to the formation of the new hydranth, are initiated by the breaking down of the endodermal ridges near the oral end of the piece. The cells of the ridges break up, setting free their contents in the fluid. Since these cells contain red pigment, as well as other products, the fluid becomes more or less filled with these granules, as well as with broken cells or even entire cells containing the pigment. There is nothing mysterious or obscure in regard to the source of this pigment in the fluid. It owes its origin to the degenerative changes preparatory to forming a hydranth. The ridges at the aboral end of the piece are also to some extent absorbed, but much less so than at the oral end, except in cases where an aboral hydranth is forming, when the same extensive process of absorption occurs as at the oral end. It is evident that the pigment observed in the circulation is a by-product in the regeneration of the new hydranth, and from its origin we get no suggestion of its being a substance formed for building up a new structure. It will also be observed that the position of the new hydranth, indicated by the degeneration of the endodermal ridges, is already determined when the red pigment appears in the circulation.

Second. As has already been stated, the pigment, along with other débris from the circulating fluid, is often collected in a ball within the forming hydranth. It appears that at this time, when the primordium of the hydranth is already laid down, the soluble parts of the broken down cells in the circulation are absorbed, presumably being digested, while the *red pigment is not absorbed but is later ejected from the mouth of the new hydranth.* Whether some part of it may not be absorbed as Loeb intimates, without however showing that the process occurs, we cannot of course state positively, but if this does happen, the amount absorbed must be inconsiderable since the entire amount appears to be ejected. Even if some of it were absorbed,—of which there is no evidence,—it would be far too small in amount to account for the pigment in the new hydranth. Loeb states that "the regeneration of a new polyp proceeds from those points where the pigment granules collect." Our observations show that the pigment granules more often collect at the distal end of the piece anterior to the tentacle anlagen. When they are seen within the tentacle region they are by no means evenly distributed over the surface, but occur in one or more irregular masses so slightly attached to the wall that they are often washed away by the current. These masses of pigment and other dêbris are sometimes carried to the basal end or to a sharp bend in the stem, remain there for a time, and later appear again at the distal end. Such being the case, the pigment granules could with difficulty be imagined to be a hydranth-forming substance.

Third. A microscopic examination of the pigment in the wall of the tube, and also in the circulating fluid, shows that it is composed of irregular crystalline-like red and yellow particles of various sizes and shapes. In addition there are spheres of an oily appearance that may represent reserve food material. These spheres also help to give a general yellowish tinge to the stem, especially to old stems and stolons, but the main color in regions of new growth is due to the red pigment. Where a new hydranth is developing, more pigment begins to appear in the endoderm and a proportionately larger number of small granules seems to indicate that this pigment is manufactured by the cells themselves. That the endoderm cells have this power there can be no question from the facts to be mentioned presently. Why, then, since this can be shown to be the case, imagine another wholly superfluous process to go on, viz: an absorption of red pigment from the circulating fluid?

Fourth. If the amount of pigment in the entire circulation be compared with the amount of new pigment in the developing hydranth, it will be found that the latter is many times in excess of the former and hence must have been formed *in situ*. This is especially noticeable in very short pieces.

Fifth. The amount of pigment set free by the degeneration of the ridges in the hydranth-forming region is approximately the same for short and for long pieces. Hence the relative amount in the circulation is far greater in short pieces, yet these, if moderately short, develop no faster than do longer pieces, and if very short it takes a longer time for the hydranth to regenerate. In the latter case, however, though not in the former, the problem is complicated by another factor, namely, the reduction in the length of the hydranth-forming region, which in itself seems to involve a delay in the development.

Sixth. The formation of new pigment is not characteristic of the new hydranth alone; but in the development of a new stolon a large amount of new pigment is formed, and at a time when there is none present in the circulation.

We may now examine in the light of these statements the arguments which Loeb believes are in favor of the red pigment being a factor in the development of new hydranths.[1]

With the following statements in Loeb's paper we cannot agree:

First. On p. 154 Loeb states, "I found further that at that end at which a new polyp forms, the formation of this organ may be recognized beforehand from the fact that red pigment granules collect at this end in relatively great density. New pigment granules are transported by a current of fluid to this end, and evidently remain lying or stuck in the neighborhood of the cut surface. The stream of fluid is kept up by ciliary movement. I expressed at that time the opinion that such a transportation of materials by the stream of fluid toward the cut end is one of the conditions of the formation of organs in *Tubularia*, and that the polarity of the *Tubularia* stem, *i. e.*, the fact that the polyp forms earlier at the oral pole, depends on the fact that the 'organ-forming' substances (possibly the red pigment granules) collect in sufficient quantity sooner at the oral than at the aboral end." We find on the contrary that the end where the polyp is to develop begins to thin out in preparation for the formation of the polyp and the primordia of the tentacles may be laid down before the red pigment begins to accumulate. Loeb speaks of the current as passing forward, and this appears to lend support to his view; but it should not be overlooked that the current flows backward on the other side of the stem and the same argument could be made to show that the aboral end should develop its polyp first, because a current also flows in this direction!

---

[1] Concerning Dynamic Conditions which contribute toward the Determination of the Morphological Polarity of Organisms. University of California Publications. Physiology, Vol. I, Nos. 16 and 17. 1904.

Second. By sticking the oral end in sand Loeb found that a larger proportion of the pieces produced aboral hydranths, and in a shorter time than when suspended freely in water. This result is cited as an argument in favor of the "assumption" that the red granules are "organ-forming substances;" but it is not clear that the experiment has any bearing on this point. Loeb appears to mean that since the red substance can not be used at the oral end, which is embedded in the sand, that, therefore, it can be used by the aboral end. If this is his meaning, it should be pointed out that the original assumption has now taken a very different form, and the polarity is accounted for not by the direction in which the current flows, but by an abundant supply of a formative or nutritive substance. With this point of view, so fundamentally different from the other, we are ready to agree, provided that the nutritive substance is something else than the red pigment. The experiment of ligating the stem, which Loeb states that he carried out "during the last summer," had already been performed by Driesch ('99) and by Morgan ('01), and the theoretical questions involved were discussed by them. Loeb states that in ligated stems, only polyps appear at the aboral end and never "roots." While this is true in general, yet occasionally a "root" develops at the aboral end in the California species of *Tubularia.* There is, however, no such sharp distinction between stem and stolon as is implied by the use of the term "root," since a stolon may produce a hydranth at its end in the same way that one is produced at the end of a stem. If the stem is ligated or has its oral end stuck into sand the stolon may produce a polyp at its distal end. A fuller discussion of this property of the stolon will be taken up in another connection.

Third. Loeb concludes that "the ligation of a piece cut from the stem of *Tubularia* results in the suspension of the polarity as far as regeneration is concerned." On the contrary we shall bring forward evidence to show that the polarity is altered mainly near the region where the aboral polyp has developed, and that a reversal or suspension of polarity does not extend throughout the rest of the stem. It is true that by ligating the stem a more rapid development of the aboral polyp is brought about, as Driesch and Morgan had already reported. Even bending the stem sharply produces the same effect, as Morgan has shown. This question of the more rapid regeneration of the aboral polyp when the oral end of the piece is closed will be fully discussed below.

Fourth. Loeb states (p. 158), "The experiments so far described had shown that by ligaturing a piece cut from the stem of *Tubularia* one abolishes the difference between the oral and aboral poles. The question arises whether one can go farther and even reverse the polarity, *i. e.*, whether one can force the aboral cut end to form a polyp sooner than the oral end. This is in fact possible, though this phenomenon does not manifest itself with the same degree of certainty as the results of the ligature experiments just described." The experiments, which he believes demonstrate this point consist first in cutting off a piece of the stem and ligating it in the middle; then after the aboral polyp has developed, the half of the stem lying aboral to the ligature is cut out, one cut end being near the ligature (the old oral end), the other near the aboral polyp (the old aboral end, but now that the polyp has appeared the new oral end). If the latter end should produce "at least in a proportion of the cases" a polyp sooner than the old oral end, this would show, Loeb thinks, that the polarity of the piece has been reversed. He found that the old aboral end (new oral end) produced a polyp before the old oral end in ten cases and in five cases the reverse occurred. From this Loeb concludes that the polarity of the piece has been reversed. Such, however, is not the case. All that the experiment really shows is that in some pieces the conditions are more favorable for the development of the polyp at the new oral end (old aboral end), where the polarity has been changed, but the rest of the piece has retained its original polarity as in the experiments in which the oral end of the piece was buried in the sand.

Fifth. Loeb's summary, which follows this experiment is as follows (p. 159): "If we correlate all these observations, we get the idea that the cause of the normal polarity which appears in the regeneration of a piece cut from the stem of a *Tubularian* is based on the condition that the circulation favors the motion of various substances in the direction from the aboral to the oral pole." There is, so far as we can see, nothing in the experiments that establishes this conclusion, and facts already known seem to controvert it.

Sixth. After giving an approximately correct account of the complete, *i. e.*, the circular, course of the circulation (p. 160), Loeb states, "It is now generally found that only those pieces form a new polyp which possess a circulation." Since the circulation takes place in all living pieces of all sizes, this statement can have, so far as we can see, no bearing on the problem. The further statement "that the collection of pigment granules at one cut end precedes polyp formation" gives an erroneous impression and reverses the real order of events. The statement "that the regeneration of a polyp proceeds from those points where the pigment granules collect is unproved and we believe incorrect. Whether, as stated, stems rich in pigment always form polyps sooner than those poorer in pigment is questionable. It is not improbable that a stem lacking in the usual amount of pigment is not in a vigorous condition, hence the slower development of the polyp. Furthermore, the oral end of the stem is usually less pigmented than the more aboral parts, yet pieces cut from the oral end of a stem produce hydranths sooner than pieces nearer the basal end.

We conclude from an examination of Loeb's argument that the evidence is wanting to show that the polarity in *Tubularia* is "to be referred to a process which is comparable as to its variety with a process of streaming in the direction of the aboral to the oral pole." Whether the formation of a polyp involves the using up of available substances in the stem which may involve a consequent diffusion of material toward the place where it is being utilized (because there is in consequence a smaller quantity of it there) will be discussed in our general conclusions; but this idea is fundamentally the reverse of the one maintained by Bonnet, Sachs and Goebel, viz: that the movement of the circulating fluid in a given direction determines *where* the development of an organ shall be initiated, and is therefore the cause of polarity.

EXPERIMENTAL RESULTS.

Experiment 1. The following data will serve to show *for this species*, the frequency of the occurrence of aboral polyps in pieces lying on the bottom of a glass dish containing sea water. The polyp was cut from the oral end and the base cut off at varying distances above the stolon.

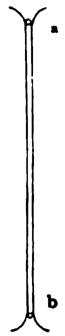


Fig. 1.

In long pieces, measuring from 10 to 25 mm. or more, about 5 per cent form aboral polyps (Fig. 1); the rest form stolons or do not regenerate at all at the aboral end. When short pieces are cut off, measuring about 5 mm., the percentage of aboral hydranths (double-headed pieces) was larger, 8.5 per cent. When very short pieces are cut off the same kind of incomplete structures that have been described for the Naples and for the Woods Hole species appear, and some of them are double structures. In some cases the new head and stolon were cut off in order to see if a larger percentage of aboral heads would develop, but this did not occur. In another series the new head only was cut off but this did not cause the aboral ends to produce hydranths; nor were they produced when only the stolon end was removed.

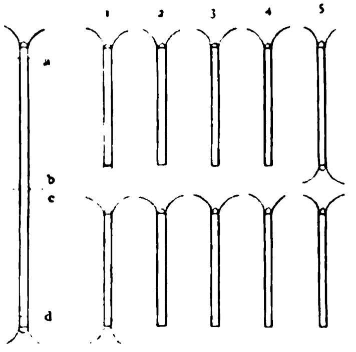


Fig. 2.

Experiment 2. In order to see what changes, if any, had been produced by the development of an aboral polyp in long pieces having an oral polyp also, the polyps at each end were cut off and the piece cut in two in the middle (Fig. 2). The pieces were kept oriented so that the different ends were known. If the formation of an aboral polyp at *d* had the same influence on the stem as the formation of an oral polyp at *a*, the middle *b c* would be a sort of neutral zone without any polarity or with a weak polar condition, if such a thing is possible. The results show that the original polar condition still manifests itself in this region, as seen in the following figures for five pieces (Fig. 2).

The aboral head at *b* in piece 5 can be accounted for without assuming it to be due to a change of polarity, for aboral heads, as we have seen, sometimes develop on short pieces. The absence of a polyp at *d* on the other half indicates that the change caused by the aboral polyp is not great. Later a polyp appeared on piece 1 at *b* and at *d*.

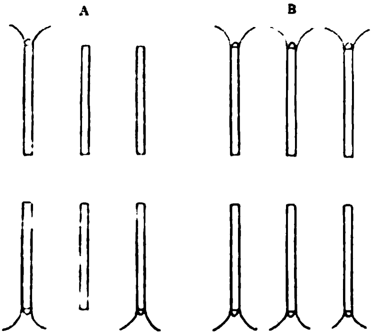


Fig. 3.

In the next series (Fig. 3), with three pieces, the influence of the aboral polyp is better seen, but the old polarity still shows itself in the oral heads. These pieces were cut June 30. Record A was taken July 2; record B, July 3.

In another series four pieces developed as shown in Fig. 4. In the first and third pieces the old and the new polarity are exhibited in the aboral half.

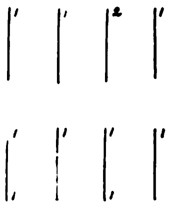


Fig. 4.

The following table gives the results for 19 pieces cut as above at different dates, from June 27 to July 24. The figures indicate the order in which the hydranths appeared. The letter *s* indicates a stolon.

The appearance of hydranths simultaneously at *b* and *c* and of stolons at *c* in several cases may indicate a more or less unstable condition of polarity near the middle of the piece; but as a whole, the table shows a stronger tendency to produce hydranths at *c* (oral) than at *b* (aboral), *i. e.*, the original polarity in most cases overbalances whatever change may have been effected by the development of a hydranth at the aboral end of the original piece.

In order to determine whether, if a neutral zone exists in double-headed pieces, it lies nearer the oral or the aboral end, several pieces were cut nearer the old oral end, and others nearer the old aboral end. The results are shown in Tables II and III.

In Table III the number of pieces which produced hydranths at *c* is proportionately much greater than in Table II. The results so far obtained might be construed to mean that the influence of the aboral polyp extends only a short distance and that its influence extends much farther in some cases than in others. Under this supposition we must account for the hydranths at *b* in Table III as in other double-headed pieces.

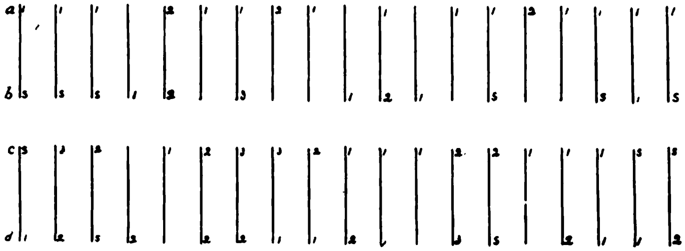


TABLE I.

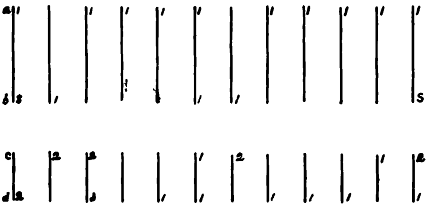


TABLE II.

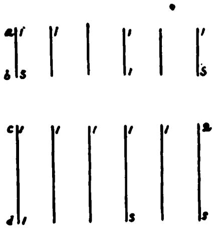


TABLE III.

Experiment 3. In this experiment the oral end of the pieces was stuck into sand and kept there until the aboral head developed at the free end. The pieces were stuck into sand July 1. After 48 hours a few pieces had regenerated aboral heads, and after 72 hours nearly all had new heads. The pieces were then taken from the sand and the new polyp (old aboral end) and the old distal end cut off. The result ought to show whether any change in polarity had occurred. After three days eight of the pieces had polyps on the old oral ends and three had stolons on the old aboral ends (where the new polyp had developed); two pieces had heads on the old aboral end; one had a head on each end, and eight had not yet regenerated. After another day the record of these pieces was as follows: Twelve had polyps on the old oral end; seven had polyps on the old aboral end (six of these had oral heads also); five had stolons on the aboral end (of these four had a polyp on the old oral end and one had none). After another day fifteen had polyps on the old oral end; eight had polyps on the old aboral end; five had heads on both ends and four had long stolons on the old aboral end. These results show that despite the fact that the oral end was stuck into sand, the original polarity of that end is markedly shown when it is cut off. It is equally clear that the development of a polyp at the aboral end has changed the condition of that end so that it is more likely to produce a polyp (when it is cut off near the new aboral hydranth) than before. Thus the polarity of the whole piece is not reversed but a new growing region is established at the old aboral end. Another point in this connection is not without interest; namely, that the end in the sand begins to form an oral polyp in this species of *Tubularia*, and the process may go so far that the hydranth is fully formed and ready to come out. It fails, however, to emerge, unless removed from the sand, and is soon absorbed. Meanwhile the aboral hydranth has regenerated. We shall consider this point more fully later.

Experiment 4. In order to show more precisely the limits of the change that takes place at the aboral end as a result of the development there of a polyp, the long pieces were cut into many short ones. In the records given in Tables IV and V double-headed pieces were used; in the second series, Tables VI and VII, the oral ends of long pieces had been stuck into sand or vaseline and kept there until the aboral polyp appeared. As, however, in most cases, a hydranth forms at the oral end in sand or vaseline, such pieces are practically double-headed pieces. The pieces were then cut up into shorter ones varying from 5 mm. to 10 mm. or more in length. The development of the shortest pieces may be somewhat retarded, that of the longest pieces little if at all. Since in each set the pieces were of approximately the same length, the retarding influence is probably constant for each series. Fig. 5 gives an idea of the way in which these pieces behave.

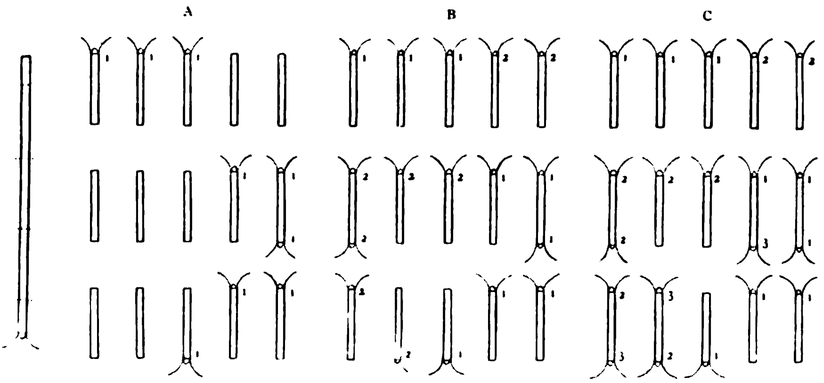


Fig. 5.

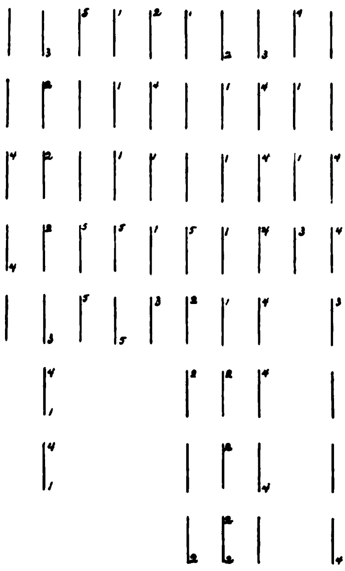


Table IV.

The remaining series are given in a different form. In each case the old oral end is at the top in the series. Those polyps that emerged simultaneously have the same number; thus the first to emerge are numbered 1, the second 2, etc., the figures being placed near that end at which the polyp formed. In Tables IV and VI the figures 1, 2, 3, 4, 5 indicate observations made once a day for five successive days. In Tables V and VII the figures indicate the sequence for

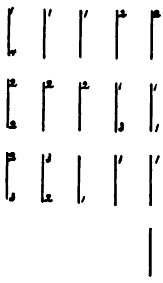


Table V.

each long piece, observations having been made three or four times daily. A figure opposite the middle of a piece indicates a double structure that did not emerge.

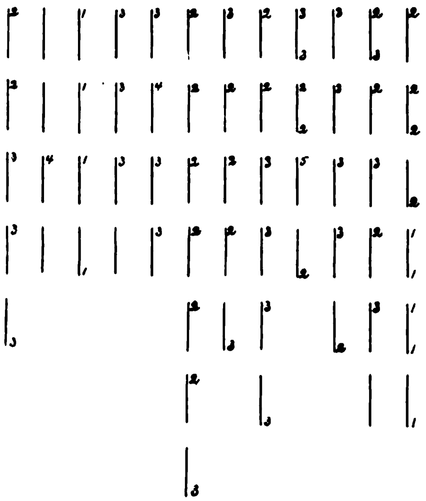


TABLE VI.

In order to contrast the development of a series of short pieces cut from unaltered stems, *i. e.*, stems with only an oral polyp, one small series of such pieces was observed with the following results (Table VII):

Here the oral ends of the pieces produce polyps, and there are few aboral polyps. It is interesting to note in the third column that something has delayed the development of the oral ends of two pieces and aboral polyps have developed instead. Whether the single case of a double-headed piece in this table (and by inference in other results when the piece is not so short that the tentacle areas of the ends overlap) can also be accounted for in this way, can not be established from the evidence, although it seems not improbable.

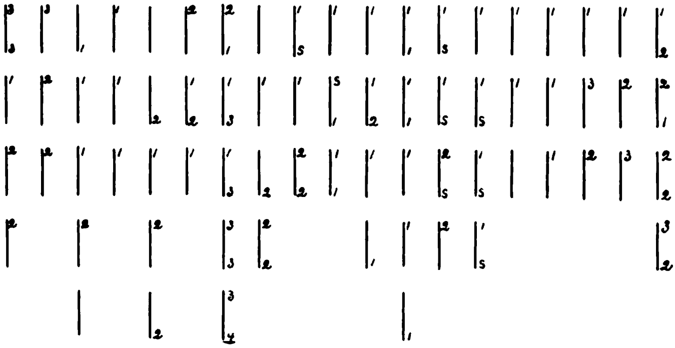


TABLE VII.

Experiment 5. The rate at which the hydranth forms is, it appears, an important factor in connection with the phenomenon of heteromorphosis. A series of experiments was undertaken to determine whether the amount of material in the circulation has an influence on the rate at which the hydranths appear. This and the five following experiments have a bearing on this point.

In several series a long piece was cut out and its ends allowed to close. Then at intervals small pieces were cut from the aboral end. Each time that a small end-piece is removed a part of the circulating fluid with its contained granules escapes from the long piece into the water. The cut end closes in the course of an hour making further escape of fluid impossible. It is true that a small amount of new material is added from the ridges at the aboral end each time the piece is cut, but it appears that not enough is added to compensate for that which is lost. The greatest amount of pigment, etc., always comes from the endoderm of the oral end where the changes preparatory to the formation of a hydranth takes place. The following are the records of the results. A check series of pieces, not cut at intervals at the aboral end, was kept in each experiment. In one set the long pieces were cut at 10.00 a. m., July 15, and at 3.00 p. m. the aboral ends were cut off. The next morning (July 16, 9.45 a. m.), 18 of these pieces showed the primordia of tentacles, two had none. The check pieces were in the same condition. At 4.10 p. m. none of the polyps was out. The next morning (July 17, 9.00 a. m.) 11 hydranths were out, 6 showed the primordia of polyps, one nothing. Of the check series, 9 polyps were out, and 3 pieces showed primordia. The following morning 16 polyps were out, and 2 not yet out; of the check set 10 were out and 3 not yet out.

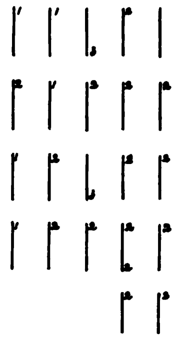


TABLE VIII.

In another set (July 31) the pieces were cut off at 11.30 a. m. The first aboral cut was made at 5.50 p. m., and the second the next morning at 9.00 a. m. At this time the beginning of the polyp could be faintly seen. At 10.00 a. m. on the following day (August 2), 6 polyps were out, 1 was coming out and 1 showed nothing. Of the check set, 4 were out and 1 had the primordia present.

In a third set the pieces were cut out at 10.45 a. m., July 30. The first aboral cut was made at 2.35 p. m., the second at 6.00 p. m., and the third the next morning (July 31) at 9.40 when the primordia were present. After another 24 hours (August 1, 9.45 a. m.) the polyps were all out in the normal time. These results show that by opening the stem and allowing the escape of fluid, the development of the polyp is not retarded.

Experiment 6. In two experiments short pieces were cut from the oral end at the same intervals as in the last series; Experiment 5. This operation suffices to let some of the fluid and the collection of pigment escape. The polyps were slightly retarded (emerging at 3.00 p. m., August 1), which is not surprising when it is recalled that the cuts removed a small portion of the distal end of the hydranth.

In another series the pieces were cut off July 14, at 2.30 p. m., the first oral cut made at 6.00 p. m., and the next day (July 15) two cuts were made at 10.00 a. m. and 3.00 p. m., respectively. The following morning (July 16, 9.30 a. m.,) another cut was made when the primordium of a polyp was present on 4 pieces. The check pieces were in the same condition. The polyps did not emerge until July 17, when proportionately as many were out as in the check series. There was a slight delay caused by the cutting, owing no doubt to too much of the polyp being removed, but since the primordia formed at the same time as in the check series, it is clear that the development up to this stage was not delayed by removing very short pieces of the oral end of the stem at four different times. Very small pieces only should, of course, be removed, for if a longer piece is removed, the region of tentacle formation is cut through, which will cause a delay in subsequent development. The most distal part of the hydranth-forming region goes to make the proboscis and may be removed in the early stages without interfering seriously with the development of the hydranth.

Experiment 7. With a fine needle it is possible to puncture the membrane over the closed aboral end of the piece and thus to allow a partial escape of the fluid. Two series of experiments of this sort showed that the oral polyp formed as soon as in the check pieces.

Experiment 8. By tying a succession of ligatures of silk thread around the aboral end it was hoped that more material might be thrown into the circulation; and if its presence had any influence on the rate of polyp-formation, the hydranths should appear sooner than in the check pieces. It was found, however, that no appreciable addition of material could be made in this way, since so little of the ridges breaks down at the aboral end. There was no hastening of the development after tying two or three times. The details may be omitted.

Experiment 9. A more successful method of introducing material into the current consists in rolling a glass rod from behind forward for a short distance over the aboral end. In this way, as Godlewski has shown, the cœnosarc may be rolled into the interior of the more anterior part, and we have found that some at least of the plug may at times enter the circulation. Two series of experiments showed that the oral hydranth cannot be hastened in this way.

Experiment 10. By crushing the aboral end it is possible in some cases to introduce a large amount of substance, including the red pigment, into the circulation. Comparing the rate of development of pieces containing a great deal of material in the circulation, with other pieces containing very little, no difference in time of polyp-formation was detected.

Experiment 11. This experiment was undertaken in the hope of determining what changes take place in the piece during the formation of the oral hydranth. Long pieces were cut out, and then at different intervals (8, 12, 18, 24 hours, etc.,) they were cut up into short pieces (5–6 mm.), which were placed in a row and the time when their polyps appeared recorded. If there is a movement forward of material in the wall, or if material in the wall is slowly used up from the oral to the aboral end, we hoped that this might be demonstrated by the time of development

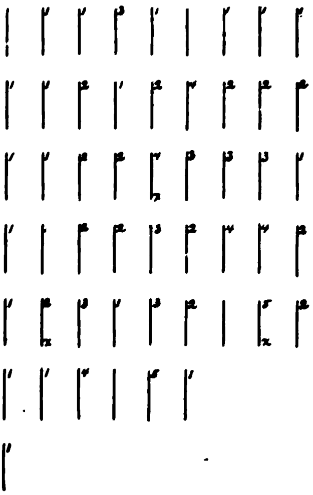


Table IX (6 hours).

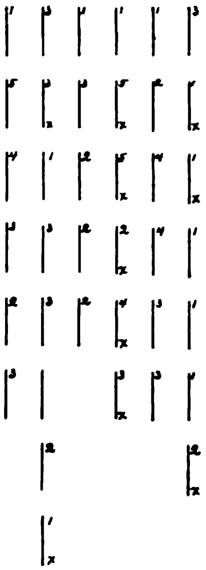


Table X (15 hours).

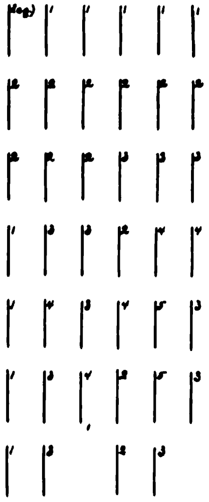


Table XI (16 hours).

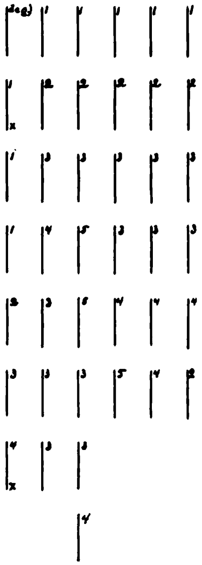


Table XII (20 hours).

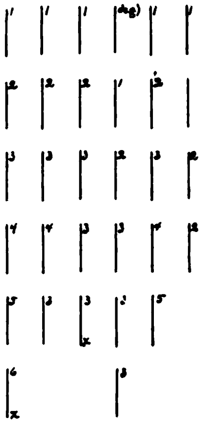


Table XIII (24 hours).

in the series of short pieces. So much irregularity occurs, however, that it is doubtful if the result establishes any definite conclusion.

In the first series the long pieces were cut into short pieces (5–6 mm.) after 6, 15, 16, 20, 24 hours. This series, kept in a warmer room, regenerated much faster than the second series. The figures indicate the sequence of the oral polyps, and *x* indicates an aboral hydranth.

After 6 hours, as shown in these tables, the intervals between the time of emergence of the polyps in succeeding pieces are not very different, although, as a rule, the piece nearest the oral end developed first. After 15 hours the irregularity is so great that no conclusion can be safely drawn. The following series, after 16 hours, shows a slightly regular sequence from oral to aboral end. After 20 hours a greater regularity is observable, and it will be recalled that at about this time the primordium of the polyp can usually be seen. Also after 24 hours the sequence is more marked.

In the next series the intervals were different, the short pieces averaged less in length and the development was very much retarded, largely due to lower temperature, as compared with the preceding series. The figures indicate observations 24 hours apart.

In the check series the pieces from the same stem developed in about the same time, although in some cases the more basal pieces were later. In the third row the most aboral piece produced a stolon and later its end developed a polyp. The 8-hour series is not good, but so far as it goes, it shows no more than the check series. The 12-hour series is not different from the preceding. The 18-hour series shows no special influence of the changes that were going on at the oral end of the piece before it was cut into small pieces. The first two rows developed very slowly. The 24-hour series developed much better, *i. e.*, earlier than the preceding, but while in most cases the oral pieces developed first, there was not a relatively great difference between these and the more aboral pieces. After 36 hours there is a decided difference shown by the oral pieces, the remainder of each set being much the same, and this is also true for the 48-hour series.

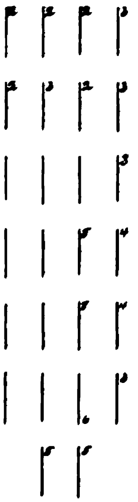


TABLE XIV (8 hours).

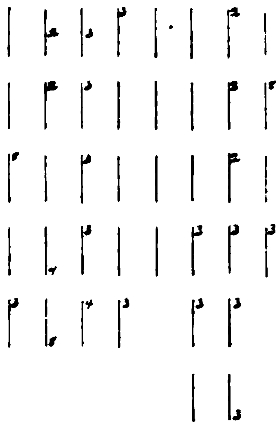


TABLE XV (12 hours).

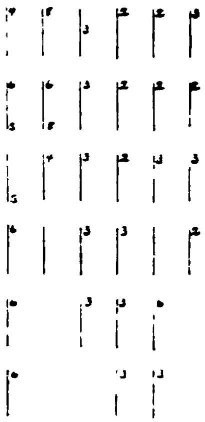


TABLE XVI (18 hours).

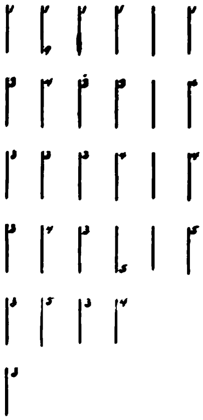


TABLE XVIII (36 hours).

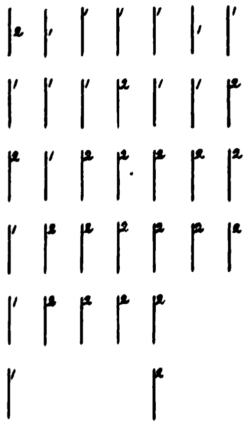


TABLE XVII (24 hours).

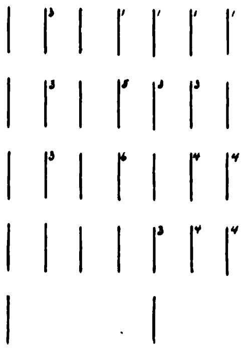


TABLE XIX (48 hours).

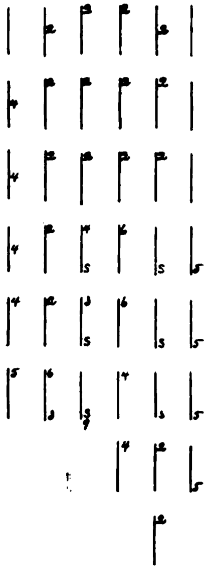


TABLE XX (check).

The second series developed slowly as has been said, owing to colder weather. Even the long pieces, kept 48 hours before cutting, had not pushed out their polyps, although the primordia had begun to appear. In the 24-hour series, the primordium was so far developed that it could be easily seen, and after 36 and 48 hours the hydranths were fully formed and ready to emerge. Whether we can correlate with this latter condition the relatively more rapid development of the pieces that lay just behind the oral region is perhaps questionable, but it appears not improbable.

The main result of the experiment is negative; for, no satisfactory evidence was obtained to show what changes are taking place in the stem during the formation of the oral hydranth, except perhaps in the case of the pieces immediately behind the hydranth-forming region in the series cut after that region was well developed. It will be noted that there are fewer double-headed short pieces in these sets than in those cut from double-headed long pieces. Since the shortness of the pieces may have caused a delay in development and thus introduced another factor, we tried the following experiment in which long pieces were used.

Experiment 12. Here long pieces were cut from the stem, and after certain intervals, the oral end was cut off at two or three different levels, and the time required for the development of a polyp was noted and compared with that in a check series in which the old hydranth was cut off at the time of the second operation on the long pieces. In one set the long pieces were kept 12 hours after the old head had been cut off, and then about 4 mm. of the oral end was cut off in some pieces, in others about 15 mm., and in others a much longer piece. Check pieces were cut at the first and last of these levels at the same time. No difference in the time of regeneration at the three levels or in the check experiment could be detected.

Other pieces of the same set were cut off after 17 hours and gave the same result. In another set the long pieces were kept 24 and 36 hours (when the primordia of the polyps had appeared) and the oral ends were then cut off at three levels, (*a*) just behind the primordium, (*b*) 10 to 20 mm. further back, and (*c*) far back nearer the base of the stem. Check series were also made. Here again no difference could be detected in the rate of regeneration at the three levels or in comparison with the check series. These results seem to indicate that the rate of development is not determined by the nearness of the new cut surface to the region where a hydranth has developed, and thus supports the view that the material that is made use of in the formation of a polyp comes mainly from the polyp-forming region and not from the whole stem (except perhaps to a slight extent from the broken down ridges from the aboral end as will be spoken of below.) It is true that pieces kept under artificial conditions regenerate after a time less rapidly than fresh pieces, but this may be due to general exhaustion of food substances thoughout the piece, so that there is relatively less material from which to produce a new polyp.

Experiment 13. The more rapid development of the aboral polyp when a ligature is tied around the stem has already been noted by Driesch, Morgan and Loeb. In *Tubularia marina* the aboral polyp so seldom develops in pieces left on the bottom of a dish that this method and that of sticking the oral end into sand or vaseline is the best way to obtain heteromorphic regeneration. Loeb found that it makes little if any difference in the time of appearance of the hydranth, how far the ligature was from the basal end. As this result has a direct bearing on our problem we repeated the experiment a number of times and under somewhat different conditions. In the first series some pieces (cut very long) were tied August 1, 10.00 a. m., near the polyp, others near the middle of the stem, and others near the basal end. Three days later one tied in the middle had an aboral polyp; seven hours later one of the pieces tied near the aboral end also had a polyp. During the four following days no other aboral polyps developed. In another series (August 1, 10.15 a. m.) after three days, one piece tied near the polyp, one tied in the middle, and one tied near the aboral end had polyps. The next day another tied near the polyp had regenerated, and the day following another tied near the polyp had an aboral hydranth. No more developed during the next two days.

In another series (August 1, 5.00 p. m.) one piece tied near the aboral end developed in two and a half days, and during the four following days no others developed.

In another set, after three days, one piece tied near the polyp and one tied near the basal end had regenerated, and three days later one of those tied near the middle developed a hydranth.

In another set, after four days, one tied near the polyp and one tied near the middle regenerated, and after three days more one tied near the basal end developed.

In two sets no aboral polyps developed.

These results, as well as other similar ones, confirm Loeb's statement that no definite relation exists between the time of development of the aboral polyp and the length of the piece of stem between the ligature and the basal end. Individual stems behave very differently, but no connection between rate and length of aboral pieces is apparent. So great is the difference in time in different cases that difference in rate of development due to another factor might be difficult to detect without more data. In this connection the regeneration of the oral polyp, which takes place more rapidly should be considered. Long pieces regenerate a little sooner than pieces 7–12 mm. long, and in shorter pieces (5 mm. or less) there is a longer delay. If a similar influence was present at the aboral end in this experiment it might not be detected. However, the length of the stem does not appear to be, in either case, the principal factor that determines the *rate* of aboral regeneration.

Experiment 14. Another series of experiments, similar to the above, was carried out in order to determine what influence the changes involved in the development of an oral polyp have on the rate of development of an aboral polyp.

(*a*) A check series of 10 pieces 15 mm. long was cut and tied in the middle as soon as cut. The oral hydranths (10) came out in from 19 to 68 hours, average 49.9 hours. The aboral polyps (9) appeared in from 46 to 76.5 hours, average 69.8 hours.

(*b*) 10 pieces 15–20 mm. long were tied in the middle 8 hours after they were cut. The aboral hydranths (8) came out on an average of 77.8 hours, just 8 hours later than those that were tied at once.

(*c*) 9 pieces 15–20 mm. long were tied 16 hours after they were cut. The aboral polyps (8) appeared on an average of 83.3 hours, 15.5 hours later than those tied at once.

(*d*) 19 pieces 15–20 mm. long were tied 24 hours after they were cut, 7 in the middle, 5 about 5 mm. from the oral end of the piece and 7 about 5 mm. from the aboral end.

The average time for the aboral polyps (7) in the pieces tied in the middle was 94.1 hours—24.3 hours more than for those tied at once. For those tied near the oral end (4) the average was 101.7 hours, and for those tied near the aboral end 102 hours —31.9 and 32.2 hours, respectively, more than for pieces tied at once.

From the results obtained in all but the last two sets of pieces, it appeared evident that it is immaterial whether the pieces are tied when cut, or from 8 to 24 hours later, *i. e.*, the changes that were going on at the oral end before the pieces were tied had no retarding effect on the aboral hydranths.

The discrepancy observed in the case of the pieces tied near the oral and aboral ends after 24 hours, was so great that the experiment was repeated twice. The results, however, were too erratic to be reliable. The peculiarities were probably due to individual differences in the stems used, as other conditions were apparently the same. Several aboral stolons were produced in both of these latter sets.

In connection with this experiment Loeb's experiment of cutting out pieces between the ligature and the aboral hydranth was repeated. In one case the oral and aboral hydranths appeared at the same time; two pieces, cut very near the aboral polyp, developed aboral hydranths and nothing at the oral end; six pieces had oral polyps and stolons or nothing at the aboral end. The evidence, though not abundant, is in favor of the supposition that the polarity of the whole piece is but slightly affected by the development of an aboral polyp.

Experiment 15. Several long pieces, which had regenerated a hydranth at the oral end and a stolon at the aboral end, had their oral ends stuck into sand without cutting off the hydranth, and after 4, 5 or more days a hydranth was formed at the uncut free end of the stolon. These hydranths were formed in the usual way and developed as rapidly as oral polyps after the primordia appeared, but a longer time elapsed before they began to form than in the case of aboral cut ends of pieces stuck into sand. All the pieces of this kind (14) produced hydranths and in several cases the hydranth degenerated and regenerated several times in the course of a month. The same result was obtained when uncut pieces bearing stolons were tied. When the stolons were cut off, a hydranth was formed very quickly at the cut end (oral) of young (recently grown) stolons; pieces of old stolons usually regenerated a hydranth at the oral end and a stolon at the aboral end.

Experiment 16. The above experiment suggested the following: Several long uncut stems were ligatured at two or three places to see whether hydranths would develop between the ligatures, *i. e.*, in parts of the cœnosarc cut off from the rest of the piece, but with the ends not exposed to sea water.

In the first set 8 stems were used and one oral hydranth developed between the ligatures after 6 days. In the second 7 stems were ligatured. One oral hydranth appeared after 7 days, one aboral after 13 days, and another oral after 20 days. The first set might have yielded further results if they had been observed for a longer time. Experiments 15 and 16 show that a hydranth may be formed at an end of stem or stolon not exposed to sea water by cutting the perisarc, but the development of such hydranths is long delayed. The absence of a hydranth, however removed, whether by natural degeneration, cutting, burying in sand or ligaturing, seems to lead to the regeneration of another hydranth, but the rate of regeneration depends on other conditions.

Experiment 17. In connection with Experiment 2, it occurred to us rather late, when little available material was at hand, that long double-headed pieces should be cut in the middle, without removing the hydranths, to see whether the two cut ends would behave alike or differently. Only one piece regenerated and that produced stolons on both cut surfaces, regeneration being most rapid at the aboral end of the distal half of the piece.

Experiment 18. Delayed development in several series of short pieces suggested the possibility that in this species light might be a factor in the rate of regeneration. To test this three sets of similar pieces were placed (*a*) in bright light near a window, (*b*) in diffuse light, (*c*) in total darkness. The rate of regeneration for the three sets was the same.

Experiment 19. The polarity of stolons was examined by means of the following experiments: Young stolons that had developed during four or five days and were stuck to the dish were cut off, and each one produced a hydranth at the cut end. Old stolons by which the *Tubularia* had been attached to rocks were also cut into pieces 5–10 mm. long and kept oriented. In 10 cases the polyp appeared on the end nearer the free end of the stolon; in 18 cases on the end nearer the stem. Pieces cut from recently grown stolons which had produced a polyp at the free end, regenerated a hydranth at the end nearer the polyp. These results show that the "polarity" of the stolon is not so marked as that of the stem. When it is recalled that the free end of the stolon may produce a hydranth if it does not come in contact with a hard surface, the behavior of the two ends of the pieces is not so remarkable. The free end of a stolon behaves in most respects like an aboral, exposed end of a stem which also, under certain conditions, produces a polyp.

Experiment 20. In order to see to what extent changes preparatory to the formation of a polyp have taken place at the aboral end of a long piece that has regenerated an oral hydranth, pieces that had within two or three days produced an oral polyp were used. The tip of the aboral end was cut off (in most cases) and the piece removed near the middle or nearer the aboral end. If changes preparatory to the formation of a polyp had taken place, we should expect the aboral polyp to develop first unless the original polar conditions enabled the oral end to outstrip the aboral end despite the advantage the latter might be supposed to have gained by exposure to sea water. The results showed that in 29 cases the oral polyp only developed, in 6 cases the aboral, in 1 case oral and aboral at the same time, and in 1 case the aboral 6½ hours before the oral. A higher percentage of aboral polyps developed than when pieces are cut out of stems that have not previously been cut, and this may be due to the changes already begun at the aboral end; but on the other hand the large predominance of cases of regeneration at the oral end indicates that the changes at the aboral end are slight, and that the original polarity of the material is still the more important factor.

AN ATTEMPT TO ANALYZE THE PHENOMENA OF POLARITY IN TUBULARIA.

BY

T. H. MORGAN.

Two questions arise in connection with the problem of polarity in *Tubularia:* (1) The *kind of structure*, polyp or stolon, that develops on a cut surface; and (2) the time at which the new structure appears. The former includes the idea of "polarity" in the usual sense; but, as I shall attempt to show, the time of appearance of the new structure may also be an important factor in determining the kind of structure that develops when an alternative exists.

A cut surface at any level may produce a polyp or a stolon. Usually polyps appear on distal cut surfaces, stolons on basal ones. An external stimulus, viz: exposure of a cut end to water, calls forth the development of a hydranth and the hydranth may develop either on a distal or on a basal surface. If a piece is open at both ends, the oral hydranth develops first, and after it has emerged, the aboral end may also produce a polyp, rather infrequently in *Tubularia marina*, but more often in *T. mesembryanthemum* and *T. crocea.* On the other hand, if the stem is tied in the middle, the basal polyp develops sooner than when the stem is not tied, and it appears in practically every instance. The aboral development takes place to a large extent independently of the length of the stem between the ligature and the aboral end.

How can we account for these facts ? We can at least formulate a provisional hypothesis. We may assume that the gradation of the material is of such a kind that the hydranth-forming material decreases from the apical toward the basal end. The formative influence, acting from the exposed end inward (the stimulus of the water on the free end), finds a prompter response when it acts in the direction of decreasing amounts of hydranth-forming material (which has the same gradation as that in the hydranth itself) than when acting in the reverse direction (namely, at the aboral end). Therefore, the oral polyp, as a rule, develops first. For its development it needs certain nutritive material. This it finds either in the cœnosarc or in the circulation, and uses the material as it develops. In consequence the cut surface at the basal end cannot get the material necessary for it to develop into a hydranth, and it either remains undeveloped or produces a stolon. If, however, we tie a ligature around the piece, the aboral end may then make use of the substances that are present in the isolated aboral part of the piece; hence there develops a hydranth. Its development is usually delayed as compared with that at the oral end of a piece cut off at the same level. Why does this delay occur? It can be accounted for in either of the following ways. It may be assumed that even when the stem is tied the oral end near the ligature often begins to form a polyp, and this developing end uses up at first all of the available food material, but as development at this end stops the stimulus of the water on the aboral end makes that end active in the presence of an excess of material. That the oral polyp may actually begin to develop or even completely develop when the stem is tied, or when one end is stuck into sand, or into vaseline, has been shown by our experiments. On the other hand it is important to note that the assumed gradation in the material from hydranth to base must be first reversed before the aboral hydranth can develop. The necessity for reversing the arrangement may in itself account for the delay in the development of the aboral hydranth, and for the prompter development of the oral one.

As has been said, the hypothesis that I advocate is, in principle, fundamentally different from that of the Bonnet-Sach's view, more recently advocated by Goebel and by Loeb. I assume that the results are not due to the movement in a definite direction of a formative or even of a nutritive material that causes the development of a hydranth. On the contrary, the hydranth begins to develop and it then makes use of the material that comes to it. If in special cases, such for instance as those where no circulation is present, the using up of material causes a movement of nutritive substances toward the place where they are consumed, such a movement is the effect of the development and not its cause.

In the case of *Tubularia* it is clear from many experiments that the rotary circulation in the stem (which is the result of ciliary action) can not be made to account for the polarity of the stem. The red pigment does not appear to be a nutritive or a formative material. It seems to bear no other relation to the regeneration than that of a waste product. The remaining material of the ridges that is set free along with the pigment may be, and probably is, a nutritive substance that is made use of by the developing hydranth.

Some other possibilities involved in my hypothesis may be briefly mentioned. Experiments have shown that when a polyp develops on the aboral end some change takes place, especially in the region immediately behind the polyp, of such a sort that we may say the "polarity" of that region has been changed. This means, on my view, that by the development of a polyp on this end the region behind the polyp has been also changed and is more like the region behind a normal polyp. Consequently a *short* piece in this region may produce an aboral polyp (in the original sense) before an oral one. How far this influence extends is not conclusively shown, but the effect is certainly stronger just behind the new aboral polyp, and it probably diminishes rapidly in the original oral direction.

A possible objection to my view may be made on the following grounds: If pieces of medium length contain enough food material to produce oral polyps then, if food stuff were made throughout the piece, there should be an excessive amount in long pieces, and aboral as well as oral polyps should develop in the latter, which is not the case. The answer to this objection is that the change in the food stuffs that makes them soluble probably takes place only, or largely, in the region where the polyp is developing and not throughout the piece. The soluble material set free at the aboral end as well as at the oral end is utilized at the oral end, because that end develops first. The actual observations suggest, if they do not prove, that the available material may be added to by the protoplasm and its food contents set free from the endodermal ridges.

It was attempted in one experiment to determine how far the preliminary changes that take place in the aboral end represent steps leading toward the development of a hydranth at that end. A very short piece was removed from the aboral end of a long piece (to make a new surface there) at the time when the oral end had just made a polyp. The long piece was then also cut in two in the middle. It was hoped in this way to find out if preliminary changes, leading toward the development of an aboral polyp, which had been going on at the aboral end, would lead to the aboral polyp developing first. It was found that usually the oral polyp developed first, indicating that little if any change had taken place at the aboral end in the direction of polyp formation. From this result it is not improbable that the slight breaking down of the ridges at this end may not be connected with polyp formation there, but possibly only with the closure of that end. Occasionally, however, the aboral end has changed so that it produces its polyp first—a result that never occurs in this species when long pieces of the normal stem are cut off.

The result of experiments with stolons is in some respects more difficult to explain, but may still be brought into line with the remaining results. When the stolon is cut off it generally produces a hydranth on that end that was nearest to the original polyp. In other words, it behaves like a piece of the stem. In quite a large number of cases, however, the hydranth appears at the apical end. This is not in harmony with my view, unless we assume that the differences in the two ends of the stolon, *especially of a very new one*, are such that local conditions (the action of water on the new end) may be stronger at times than the differences in the two ends. Moreover, it is not to be forgotten that a stolon, not in contact with a surface, tends to produce a hydranth on its *closed* end; especially if the oral hydranth is suppressed or the oral end of the stem closed. The newness of the growing tip may also make it more responsive to the action of water if it is not in contact with a surface.

A postulate of my view is that the polyp after it has formed continues to use up nutritive material as long as it grows, and hence tends to hold in check for some time after its first formation the development of a polyp at the aboral end. The polyp when first formed is small, and can, in fact, be seen to enlarge for several days after it has emerged.

I have assumed that the stem of *Tubularia* is not homogeneous, but that from the hydranth to the base there is a graded difference and this gives the order or stratification of the material. The stimulus of the water acting on the free end arouses the formative changes which act in the direction of this existing material order. How does such a view differ from the old assumption of a "polarity" in the material? On my view there is no such directive force residing in the material as the term polarity suggests, but the polarity is only a name for the gradation of the material and on this as a basis the formative changes are carried out.

Conversely a similar gradation from the stolon to the hydranth must exist in respect to the tendency to produce a stolon, hence the latter appears on the aboral end; but in the case of *Tubularia* we find that the action of water on a free end, whichever this may be, has a stronger tendency to call forth a hydranth than a stolon in some species. Hence the conflict of influences that probably goes on at this end. If, however, the aboral end is brought in contact with a solid body a stolon develops promptly, while the oral end, if brought into contact with a solid, fails to produce a stolon, but develops a hydranth.

In conclusion, I think, that the Sachs-Bonnet hypothesis of the *migration* of formative stuffs is not needed to explain the results in *Tubularia*, and furthermore that there are no observed facts or experiments that make this view probable. I have no wish to deny that substances having a formative influence on growth may exist, but there is no evidence in favor of the view that *in multicellar forms*, such substances migrate in definite directions and thus produce the "polarity." If development begins in a region, there may be a movement of the materials that are being used up toward the developing part, but to fail to distinguish between this point of view and the former is to confuse cause and effect. The two points of view are diametrically opposite, and no good end can be reached by ignoring this fact.

The Relation Between Normal and Abnormal Development of the Embryo of the Frog: V. As Determined by the Removal of the Upper Blastomeres of the Frog's Egg.

By

T. H. Morgan.

With Plates IV and V.

Eingegangen am 5. August 1904.

The appearance of the embryo[1] of the frog on the black hemisphere of the egg in certain forms of abnormal development led me to suspect that the material out of which the normal embryo is formed may be contained at first, in part, in the upper hemisphere, and become transferred during the early development to the equatorial regions. In still later stages a large part of the embryo-forming materials is carried from the equatorial regions over the lower hemisphere of the egg by the advance of the lips of the blastopore. It is the former transfer of materials, and not the latter, that I propose to examine here in the light of certain experimental results that I have obtained during the spring of 1904.

If the material to form the embryo lies at first in the black hemisphere of the egg it may be in part contained in the upper four blastomeres of the eight-cell stage, and if these should be removed the formation of the embryo should be in large part prevented, — provided the remaining parts of the egg have not the power to regenerate the material that has been removed.

By means of the gray-crescent it is also possible to locate, with a fair degree of probability, the two anterior and the two posterior of the upper four blastomeres of the eight-cell stage. If my hypothesis is correct, it should be possible, by removing the two anterior or the two posterior blastomeres, to bring about corresponding defects in the embryo.

---

[1] That is the medullary plate and the parts immediatly below.

In a preliminary set of experiments, carried out in order to see if the operation were feasible, the upper four blastomeres were punctured with a cold needle, and their contents caused to exude as an exovate. The outcome was variable. If great care was taken to remove completely all of the material of the blastomeres the remaining parts of the egg generally failed to produce an embryo, and died without proceeding very far in their cleavage. If the utmost care was not taken in removing the blastomeres, defective, or even nearly normal embryos of many kinds were obtained. These results, while puzzling and discouraging, led me to examine more critically the immediate results of the operation itself. The outcome showed that it is much more difficult to remove completely the entire contents of the upper cells than appears to be the case at the time of the operation. If the egg is given a half hour, or thereabouts, to recover, it will be found in many cases that parts of the upper blastomeres have been left behind, and since these subsequently divide, in most cases, it is certain that their nuclei have been left, and that their material has been contributed towards the formation of the embryo. After this had become evident I generally separated the eggs that had been operated on into two lots, placing in one lot all of those in which the contents of the injured blastomeres had been entirely removed, and in the other lot those in which the operation had been only partially successful.

In other cases I made use of a hot needle. The blastomeres are more certainly killed by this means; but injury to the surrounding parts of the other blastomeres may also easily occur. The hot needle was especially successful when it was desired to kill all four of the upper cells at the eight-cell stage — an operation, which, as I have said, gives very unsatisfactory results with a cold needle.

There is another consideration that can not be too strongly emphasized. Even if it is true that a large part of the material that goes to form the embryo is contained in the top of the egg, it does not necessarily follow that this material is all contained in the upper four cells of the eight-cell stage. An examination of the eggs shows that these cells have such different forms in different eggs, that they must include in some cases less, in others more of the material around the pole. It has also become clearer in recent years that cleavage-boundaries need not coincide exactly with the organ-forming areas of the protoplasm. Far from corresponding to the diagrammatic figures in most text-books, in which the four upper black cells of the frog's egg are represented as being equal in size, and placed symmetrically around the upper pole, the cells are rarely or never of equal size, and their relation to the anterior and posterior sides of the egg, and even perhaps to the upper pole of the egg itself is variable. In Figs. 1 and 2 an egg is drawn in which the upper four cells are narrower than usual from side to side, and are longer in an antero-posterior direction. In nearly all cases I have observed in this species of frog, *Rana palustris*, that the two anterior cells are more pointed, as is also to be seen in this figure. The pointed ends may extend into or even through the gray area, and in extreme cases as in Fig. 2 into the lower hemisphere as far down as that part of the egg on which the dorsal lip of the blastopore appears. In Fig. 3 the top of another egg is shown in which the upper cells are somewhat broader. In this case the whole upper group appears to be shifted somewhat in the direction of the anterior side of the egg, i. e., the meeting point of the four upper cells lies anterior to the black pole of the egg.

The results of the experiments may be considered under the following headings:

I. Removal (partial or complete) of the two anterior of the upper four cells.
II. Removal (partial or complete) of the two posterior of the upper four cells.
III. Removal (partial or complete) of the upper four cells of the eight-cell stage.
VI. Injuring the top of the egg at the two-, and four-cell stages.

I. Removal of the Two Upper Anterior Blastomeres of the Eight-Cell Stage.

Some embryos that were obtained by means of this operation are shown in Figs. 4 to 11. The variety of forms that may result is evident from this series, and without a further examination of the results of the operation there may seem to be no connection whatsoever between the removal of a part and the kind of embryo that results. When, however, the operation itself, with all of its possibilities, is taken into account the results are more orderly than appears at first sight. These embryos may now be described in detail, and then, using these as examples, the general results of the operation, as seen in other experiments, may be considered.

The embryo represented in Fig. 4 shows the posterior end of the medullary folds with a large yolk plug in the middle of the posterior region. At the anterior end the surface is raised in rounded masses, on which the head end of the embryo does not appear. Sections show that the medullary folds are united for a short distance behind and in front of the yolk plug. At each side of the yolk plug a deep half-archenteron is present. At the anterior end the brain region is undeveloped.

The embryo represented by Fig. 5 is somewhat more than a half-embryo, especially anteriorly, and lies along the right side of the exposed yolk. At the posterior end the other, left half of the embryo is somewhat developed, although anteriorly the medullary fold is entirely absent on this side. No sections were made of this embryo.

The embryo drawn in Fig. 6 shows a nearly entire medullary plate along one side of an exposed yolk mass. Towards the posterior end, however, the half of the medullary fold on the side towards the yolk is less developed. Sections of this embryo show that a nearly bilateral medullary plate is present at the anterior end, although smaller than normal. In the middle and posterior regions the medullary plate is little more, if at all more, than a half-structure with the archenteron opening at the yolk side. The opposite side of the egg — across the yolk — has been largely killed by the needle, especially superficially, and has failed to produce the missing half. It is evident that the operation has produced (as in the last case also) a greater defect on one side than on the other, and that only one side has developed, except in the head region where more than a half structure is present.

The next embryo, Fig. 7, is nearly normal, except in the head region, where the medullary folds are much less developed than in the middle and posterior regions. The effect of the injury to the anterior blastomeres has been to produce a defect in the anterior end of the medullary plate.

The embryo represented in Fig. 8 shows a different result of the operation. What appears to be a whole, although shortened, medullary plate lies on one side of the exposed yolk. Sections show that the groove on the surface corresponds to a thickening of the inner layer of the ectoderm, but whether, as appears in surface view to be the case, more than half of the medullary fold is present or only a half is not clear. At the edge bounding the yolk an archenteric invagination is present in the yolk, and a similar invagination is also present on the opposite side of the yolk mass (below the left side of the yolk of the surface view). This is only present along the posterior end, and not further forward, and here the surface appears injured and vacuolated. At the anterior end, in front of the yolk-plug, the anterior part of the medullary plate extends somewhat over to the other side, but the material is very little developed, and quite irregular in arrangement.

Fig. 9 shows again a half embryo with an imperfect anterior end, where a protrusion of the material is present.

Fig. 10 is likewise a lateral embryo. Sections of this embryo show both medullary plates present for a short distance around the posterior end of the yolk. On one side the plate quickly ends, while on the other, as shown in the figure, it is quite well developed. A large exovate is present along one side (seen protruding from below and to the right in the figure), but it is no longer attached to the egg. At the head end the medullary plate, while not much more than half — if at all so —, is more symmetrical, and forms more nearly a whole structure. It is, however, little developed.

Fig. 11 shows what appears to be a half-embryo, defective in the head region. Sections show that the other half of the embryo is also present on the opposite side of the yolk, but it is less well developed. On both sides, the head ends of the medullary plates are defective; so much so, in fact, that they may be almost said to be absent. On that side of the egg not shown in this figure, the anterior end of the medullary plate protrudes and is somewhat injured. The ectoderm contains large scattered cells interspersed with smaller ones.

In all of these embryos there is direct evidence of defects in the anterior end. The half-embryos and spina bifida embryos are due to the material that goes to form the lateral lips of the blastopore on one or on both sides failing to cover over the yolk-hemisphere, and becoming differentiated largely *in situ*. It is interesting to note also, that when one side is less developed, the posterior end of that side is better formed than the anterior, and it was the anterior material that was operated upon in this series.

Several eggs were killed at 5, 10, and 20 hours after the operation in order to find out their condition at these times. Two eggs, after 5 hours, show the blastomeres further divided and the segmentation cavity appearing. The protoplasm of the cells, that had not been themselves stuck, is drawn out towards the place of injury, and is, in consequence, somewhat injured, i. e., it is not segmented regularly, but contains nuclei and elongated pigment streaks. Four eggs, after 10 hours, show further segmentation; the segmentation cavity is large in some eggs, smaller in others, and irregular in shape. The place of injury appears to lie at a level with the floor of the segmentation cavity; and here the protoplasm protrudes somewhat and is irregularly and incompletely divided into cells. This lateral (anterior) location of the exovate indicates that the protoplasm of the remaining cells has been drawn out when the blastomeres were removed, and that a shifting of the cells remaining at the top of the egg has to some extent taken place. One of these eggs had been so much injured that it had divided in only a small region, — the rest of the egg containing a few scattered nuclei. No segmentation cavity is present.

After 20 hours two eggs show further segmentation. A large segmentation cavity is present, situated somewhat excentrically, and the protoplasm, in the region of the blastomeres that had been removed, is abnormal in appearance and not divided. Another egg of this set is much more injured, and the yolk less divided. A small segmentation cavity is present, situated excentrically.

In one set, in which the two upper anterior blastomeres of the eight-cell stage had been stuck with the intention of removing them, a few eggs were preserved at once, and later more carefully examined under the microscope in order to see how successful the operation had been. In six cases the contents of the two anterior blastomeres had been entirely removed, and the two posterior left uninjured. In one case one of the two upper blastomeres had been entirely removed, and only a part of the other one. After five hours, six other eggs were killed with formalin and examined; two of these had continued to divide, while four appear wrinkled on the surface and the protoplasm had remained undivided.

These observations give some idea of what can be expected from the operation unless each egg is carefully examined after the attempt to remove the blastomeres. While, in general, it may be said that the operation can be successfully carried out, this is not always the case, and pieces of the injured blastomeres must sometimes remain, and in consequence some portion of their material may be contributed towards the formation of the embryo. Not infrequently also the rest of the egg is so much injured by the removal of the upper blastomeres that it may fail to segment entirely, or in part. Sections show that the protoplasm of the lower cells in the immediate vicinity of the ones removed is often drawn out and may be so injured that it fails to segment; and thus a more extensive region than the part immediatly stuck may become involved in the operation.

An examination of all the material resulting from this operation gave the following results:

In one lot of eggs the posterior parts of the medullary plate were present, and the anterior part was injured or defective in sixteen cases; one normal (?) embryo developed, and three embryos had a circular blastoporic rim, but had not further developed. Eighteen eggs were dead.

In another lot two half-embryos developed, one a much shortened and imperfect half; the other an elongated spina bifida embryo; three eggs were dead. The condition of the head end in the former two was not sufficiently examined, since sections were not made.

In another lot (after 3 days) twelve eggs had segmented, but the blastopore lip had not yet appeared. The eggs showed marked injuries in the region of sticking; eight eggs were dead; four showed the beginning of the blastopore just below the black area. The formation of the embryo had been delayed in these eggs, as usually happens after an operation.

In the two following series the eggs were operated upon with greater care, and an hour or more after the operation they were re-examined, and separated into two groups; in one of which the contents of the two injured blastomeres had been completely removed, while in the other group the operation had failed to remove completely the contents of one or of both of the injured blastomeres.

Group 1. Contents all out. Six eggs had not developed further than the early segmentation stages and were dead.

Group 2. Contents not all out. Three eggs showed the posterior end of the medullary plate, while anteriorly they were defective. Two were segmented on one side only.

A few other series were also examined, but as they gave nothing different from those described above they may be omitted here.

Conclusions: The effect of this operation shows unmistakably that the anterior end of the embryo is generally defective after the removal of the two anterior blastomeres. The frequent appearance of half-embryos probably means that the egg has been more injured on one side than on the other, and it is interesting to find in these cases also, that the head end of the half-embryo is often defective. The spina bifida embryos result from the failure of the halves to grow over the yolk, and these, too, generally show a defective anterior end.

II. The Two Upper Posterior Blastomeres Removed at the Eight-Cell Stage.

An embryo from an egg operated on in this way is shown in Fig. 12. Posteriorly it terminates in a plate, projecting over the yolk, that carries on its surface a continuation of the medullary plate. In this case the dorsal lip had appeared, no doubt, and had advanced for a certain distance over the lower hemisphere, receiving contributions from the lateral lips. Its further advance was hindered by the lack of power to advance of the lateral lips, represented in part by the line between the black and the white. Sections show that the anterior end is well developed; a wide archenteron is present beneath the dorsal surface.

The next embryo, Fig. 13, is very similar. The anterior end is present, but posteriorly the embryo ends as in the last case. One side is more complete than the other, so that the medullary plate appears to lie excentrically on the yolk. This condition is due, as has been said, to one side being better developed, and, therefore, more extensively covered by ectoderm, and secondly to the greater advance of the dorsal and lateral lips of the blastopore on this side.

The next embryo, Fig. 14, is similar in many ways to the last. The head end is well developed. Sections show that while both sides are defective posteriorly, one side is more so than the other. The archenteron comes to the surface along the black edge shown on the left side of the figure. The posterior end of the medullary plate is more complete on the right side of the figure.

The embryo shown in Fig. 15 is a spina bifida form. It is not possible to determine with certainty which is the anterior and which is the posterior end. Sections show that the half-medullary plates encircling the yolk meet at what appears to be the anterior end, while at the other end, presumeably the posterior, the half-folds fail to meet, and the region between them is injured.

The next figure, Fig. 16, is little more than the dorso-anterior part of an embryo. The suckers (adhesive glands) appear at the anterior end of the black cake-like part of the egg, and behind them the groove on the surface indicates the line of union of the anterior portion of the medullary folds. The medullary plate has rolled in, and its sides have met and fused beneath the surface. Beneath the medullary tube there is a notochord in the middle and in the more posterior parts of the embryo, and beneath this is a rather large (especially anteriorly) archenteron, which extends to the posterior end, but does not open laterally along the edges of the black region. These edges, shown in the surface view, can not, therefore, be looked upon as the undeveloped lateral lips of the blastopore.

The relative number of the different kinds of embryos resulting from killing the two upper posterior blastomeres is indicated by the following records.

In one series, in which the contents of these blastomeres had been completely removed, when examined, ten hours after injury, two eggs had segmented, except in the region of the exovate; and in another lot, in which the contents of the injured blastomeres had not been entirely removed, three had segmented except in the region of injury, and one had segmented only very imperfectly.

In another series, killed four days after the operation, one egg showed a blastoporic rim on one side about two-thirds the distance down the egg; the other side of the egg seemed to be dead. Another egg had produced a half-embryo on one side (rather imperfectly formed) and an imperfect edge along the other side of the yolk. In another lot, three eggs were dead, one had formed a nearly perfect embryo, and the third is shown in Fig. 16.

In another, better series, eight embryos had a nearly normal anterior end; five eggs had segmented, but no embryo had yet developed, and eleven were dead. The embryos represented in Figs. 12—15 are from this set.

Conclusions: The evidence in favour of a posterior defect in the embryo, after the removal of the two posterior blastomeres, is much less satisfactory than in the case of the removal of the two anterior ones. We can at least surmise the cause of this difference. In the first place the two upper posterior blastomeres are relatively higher up on the egg than are the two upper anterior ones. Hence the material contained in them is probably not carried so far down on the posterior side of the egg as is the material of the two anterior blastomeres. In the second place, the posterior lip of the blastopore appears to come in further down in the lower hemisphere than dees the dorsal lip. In the third place the posterior end of the embryo is much less characteristic and difficult to identify than is the anterior end. It does not differ much, superficially, from the lateral posterior parts of the medullary folds, and if the latter should fail to develop as far as the real posterior end, it would be difficult to distinguish between their ending and the normal posterior end of the embryo.

From these considerations it follows that it would be much more difficult to establish that the two upper posterior blastomeres contribute to the embryo than was the case for the two upper anterior blastomeres. It can not, perhaps, be claimed that the results here recorded demonstrate that the material of these posterior blastomeres is really added to the embryo, although, I think, the results do make this view probable. This same question will come up again for consideration in the next experiment, in which all four cells at the top of the egg were injured or removed.

III. Injury to or Removal of the Four Upper Blastomeres at the Eight-Cell Stage.

If this experiment could be successfully carried out it ought to give a more complete answer to our problem than either of the two preceeding experiments. Certain technical difficulties, however, make it very difficult to perform the experiment with entire success. If the contents of the upper four cells is entirely removed the rest of the egg often collapses, or more often fails to develop further, although, as sections show, the nuclei may continue to divide. In the second place it is by no means easy to remove entirely the contents of all four blastomeres without causing the changes just mentioned. Nevertheless, if the contents are even largely removed the experiment should give results that bear directly on the present question, and such has proven to be the case.

Fig. 17 shows an embryo from which the four upper cells had been more or less completely removed. The top of the egg still contains an injured region. The blastopore had formed below the equator of the egg, but had not developed further, although the embryo had been kept for several days. Sections show that the whole top of the egg has been destroyed — more extensively so than the figure indicates. Beneath this dead part is a fluid space containing a coagulated fluid. The lower portion of the egg is divided into cells and the blastoporic rim, seen in the figure, is represented by a slight in-pulling of yolk cells as in the normal invagination. On one side a little ectoderm is differentiated above the blastoporic rim, and underneath this there is a little mesoderm. On the opposite side no such differentiation has taken place. The sections give every appearance that the region of the blastopore is lacking quantitatively in the material that goes to produce the embryo.

The next figure, Fig. 18, is similar to the last. Sections of this egg also show a very much injured region at the top of the egg. The lower part is cellulated, and a narrow depression marks the blastoporic rim. The cells bordering this groove are pointed and have pigment in their outer ends. There is no ectodermal or mesodermal differentiation above the rim.

In other experiments a hot needle was thrust into the upper four cells, and moved about, so that these cells were injured or killed by the heat. This method has the advantage that the egg is less likely to collapse than when the blastomeres are removed. It has the disadvantage that it is more difficult to determine after the operation how extensive the injury has been. In fact the heat may often injure the upper parts of the lower four blastomeres.

The egg drawn in Fig. 19, is a late stage after an operation of this sort. The top of the egg is dead, the lower part is cellulated. As yet the blastopore has not appeared.

Fig. 20 represents another egg in which, as sections show, a much less extensive injury than in the three preceeding cases has taken place; and, in fact, some of the material of the top on one side appears to have been left and to have formed a solid group of small cells on that side in which the blastopore appears on the surface. The latter lies in the large cells far below the collection of small cells. The larger cells show some differentiation above the blastopore.

The egg represented in Fig. 21 is similar to the last, but has developed only on one side. No sections were made in this case.

A few other eggs killed at different times after the operation were also cut into sections. One egg, after eight hours, shows the top killed, the rest divided into cells; another after ten hours shows the protoplasm undivided, the nuclei dividing; another after twenty hours shows the lower portion divided, the upper parts of which contain smaller cells than the lower; and another egg of the same lot was so much injured by the heat that the nuclei are dividing in only one part of the egg.

In the two following cases the contents of the upper four cells had been entirely removed with a cold needle. One of these, killed after ten hours, showed numerous nuclei, but the protoplasm had not divided; the other, after three days, showed the top of the egg entirely killed, the rest of the egg being divided for the most part into many cells. On one side no protoplasmic division had taken place. Later stages of eggs operated upon in these ways gave the following general results:

In one series (hot needle) ten eggs were dead; two others had segmented on one side only. In another set about sixty eggs operated upon were dead and only two or three showed late segmentation. In both of the preceeding sets a hot needle had been used and the eggs were preserved after three days. In another set (cold needle), after four days, five eggs were dead, and two had segmented only on one side. In another set (hot needle), in which the entire contents of the cells was removed, four eggs died, and two had segmented in the remaining part. In another set, in which the blastomeres were not completely killed, two eggs show a blastoporic rim, one had formed a normal embryo, and one had gone no further that a late segmentation stage.

In another set, in which also the contents of the upper blastomeres was not entirely removed, nine eggs died, four had segmented, but no embryo had formed, and two showed a blastoporic rim. In one the rim lay below the equator, and in the other high up on the side of the egg.

In still another series, in which the contents of the blastomeres had not been entirely removed four were dead, four had segmented on one side, two showed a blastoporic rim (one of these had an incomplete anterior end of the medullary plate). In another set, a blastoporic rim and the imperfect anterior end of an embryo was present.

These examples will serve to illustrate the outcome of this operation. There were more than fifty other cases similar to these.

Conclusions: The most common result of the operation is either to kill the egg or so to injure it that the other cells fail to divide, and the egg dies. When, however, the remaining portion of the egg segments it may produce a circular blastoporic rim. In some cases in which the top cells were not entirely killed or removed a part of the embryo appeared, but it is imperfect and deficient. The lack of development may be due, in some cases, either to the injury preventing the formation of the embryo, or to the lack of embryo-forming material. Both factors are, no doubt, sometimes involved, but it seems more probable, in the light of other results with injured eggs, that the outcome is due mainly to the lack of material.

IV. Injuring the Top of the Egg at the Two- and Four-Cell Stages.

In order to determine whether the material that takes part in the formation of the embryo is already present at the top of the egg in the two-, and four-cell stages, and does not move into that part during the formation of the upper four cells, the following operation was attempted. The top of the egg was stuck with a hot needle at the two, and at the four-cell stage. At the two-cell stage it is more difficult to locate the top, and the results are less uniform than for the four-cell stage. In one set (after five days) of the four-cell stage series, three eggs died; three segmented, but formed no blastopore; two had a blastopore and a trace of the anterior end of the medullary folds. Another embryo of this lot is shown in Fig. 22. The anterior end is well developed, the middle and posterior parts extend along one side of the yolk. In this case the injury has affected mainly one side, so that something more than a half embryo results. The particular conditions that lead to the formation of this kind of embryo will be discussed later. In another case a short, but nearly normal anterior end of an embryo developed.

In another series, stuck at the two-cell stage, twelve eggs showed delayed segmentation; one showed the blastopore nearly closed and a medullary plate imperfectly outlined in front of it. Another egg showed a shortened embryo lying along one side of the yolk, somewhat as in the last figure; a third had a blastoporic rim around the equator; and a fourth had a small yolk plug, but no medullary folds had as yet appeared.

Conclusions: The results of injuring the top of egg at this time give, in general, results similar to those after injury at the eight-cell stage, but the outcome is so variable that the experiment is unsatisfactory. The results recall the early experiments of Roux, in which parts of the unsegmented egg were caused to exude by sticking the egg with a needle. The many different kinds of embryos that he obtained can, with difficulty, if indeed they can at all, be referred to the kind of part removed. The shifting that takes place after an operation of this sort, if it is a severe one, causes such extensive changes in the protoplasm that the results are difficult to interpret. The same danger, but to a much smaller degree, is also met with when the eight-cell stage is punctured.

One of the peculiar methods of development that occur after injury to the egg calls for special comment. A medullary plate, that is more than a half plate, especially at its anterior end, may appear lying excentrically on the dark part of the egg. It sometimes appears to lie along one side of the yolk as shown in Figs. 6, 8 (?), 13, 14, 22. At first sight it appears that one half of the egg has formed nearly a whole embryo; and as such cases seem to occur more frequently when the other half of the embryo has failed to develop, this conclusion might seem all the more probable. The real meaning of embryos of this kind is best understood by means of the following diagrams, Figs. 23—26. The first of these shows the egg as seen somewhat from below. One side of the egg, indicated by the cross-lines, has been injured, but the injury does not extend to the middle of the region in which the dorsal lip of the blastopore appears, as shown in this figure. In later stages the lateral lip of the blastopore extends around one side of the yolk, as shown on the right side of Fig. 24, while on the other side it extends only to the region of injury. The dorsal and lateral lips now begin to grow over the lower hemisphere, as shown in Fig. 25, and finally extend to the posterior region of the embryo, as shown in Fig. 26. The medullary fold appears, as shown in this figure, on the region overgrown (and also somewhat in front of the first position of the dorsal lip). The archenteron opens to the exterior between the letters *B*—*P* in Fig. 26, but the dark-white line anterior to this, *R*—*B* in the figure, is not a blastoporic rim, but the line between the uninjured and injured parts of the egg. The medullary plate is a whole structure at its anterior end because it is derived from the material in front of the first position of the dorsal lip, where the »whole« primoidium of this part of the embryo is present. Behind the head region, the medullary plate is a complete half-structure on the right side, because it contains all of the material of that side of the egg; but the left half of the plate is incomplete, the more so the more posterior the region examined. The cause of this deficiency on the left side is due to lack of material; and that part of it that is present is due to the little material of the left side of the dorsal lip that is carried backwards as the dorsal lip grows posteriorly. The amount of this material continually decreases from before backwards, and, in some cases, may be totally lacking at the posterior end. The opening of the archenteron along the edge of the more-than-half plate, between the letters *B—P* in Fig. 26, supports the interpretation that I have given to these embryos. On any other interpretation the opening out of the archenteron at the side of a medullary plate, that is more than half, is difficult to explain.

Discussion of Results.

These experiments have been only partially successful owing to the difficulty of the operation and to the great mortality amongst the injured eggs. Nevertheless I believe the results show evidence of a direct connection between the removal of the blastomeres at the top of the egg and definite defects in the embryo. Whether these defects are entirely due to the removal of the material of the upper cells or to certain indirect effects of the operation will bear closer examination. The following considerations should be given due weight in this connection.

First: The fact that a large percentage of eggs die without forming an embryo makes it somewhat hazardous to draw conclusions from those that do survive. This objection curries less weight, however, if it be remembered that the eggs have been sorted into lots according to the extent of injury to the punctured cells, and embryos have been obtained from all the lots. This shows that some at least of the eggs that survive have had the entire contents of the injured blastomeres removed, and have produced defective embryos.

Second: The difficulty of completely removing the contents of the blastomeres affects the value of the results to some extent, unless great care is taken to examine the eggs after the operation, and to remove those, in which parts of the punctured blastomes have been left. In the cases in which the greater part of the blastomere has been removed, the effect should be almost the same as when the entire contents is removed, provided the result depends on the loss of the protoplasm rather than of the nucleus; and this, in fact, appears to be the case from the results of recent experiments on other forms, as for example the ctenophors (Driesch and Morgan, Fischel), the mollusks (Crampton, Wilson), the sea-urchin (Driesch, Boveri), etc.

Third: The effect of removal on the neighboring blastomeres, causing them often to remain undeveloped or to protrude at the region of injury, may produce a more extensive injury than that intended. This is, I believe, the most serious objection to the experiment. Sections show that this result not infrequently occurs. I do not doubt that some of the defective embryos have arisen in this way, and since the regions indirectly affected are in that part of the egg where the expected results of the operation are looked for, too great caution can not be used in interpreting the results.

Fourth: As has been already pointed out, other results led me to suspect that the embryo-forming material of the frog's egg lies, at first, somewhere around the top of the egg, though not necessarily immediatly over the pole itself. It is not probable that this material is contained entirely in the upper four cells of the eight-cell stage, and even if in large part the cell boundaries need not correspond exactly with embryo-forming areas. In fact, I believe, the recent results referred to above only show that a large amount of a particular substance may be contained in a given cell, and, in consequence, determine its fate. Other, neighboring cells especially, may contain smaller amounts of the same substance (combined with other kinds), and the kind in largest amount may determine the differentiation of the cell. From this point of view we can better understand the regenerative capacity of certain eggs after the removal of the blastomeres. In the frog's egg the different shapes of the upper cells at the eight-cell stage would seem to indicate that a larger amount of certain parts of the embryo-forming-material is contained in them in some cases and a smaller amount in others; and this, in turn, might lead to quantitative differences in the effect of their removal. Also, if I am right in my conjecture, viz., that the upper meeting point of the first four cells may lie at different points in the upper hemisphere, this also would lead to some differences in the results of the operation.

Fifth: I have tacitly assumed that the median plane of the embryo may be expected to agree with the plane of symmetry of the gray crescent. This is generally, but not always, the case, as shown by the observations of NEWPORT, PFLÜGER, ROUX, KOPSCH, MORGAN, and MORGAN and BORING. It has been shown by the latter writers for the species of frog used in these experiments, *Rana palustris*, that in a small percentage of cases the two planes do not coincide, and furthermore that the second furrow may sometimes coincide with the middle of the crescent, in which case the middle plane of the embryo sometimes agrees with the first, sometimes with the second plane of cleavage. It is, therefore, always possible that a few of the eggs may be in these conditions, and, consequently, the removal of the two blastomeres on the crescent side may not involve the removal of the two anterior, but of two lateral cells. Some of the results may be due to this, but the chance is small that such eggs are used, and develop, and the general results are not, I think, seriously affected by this exceptional relation.

Due weight must be given to all of these points. I have kept them constantly in mind, but I think that serious as the difficulties are, the results indicate a direct connection between the operation and the effect produced in the embryo, and I conclude that the removal or the destruction of the material of the upper four cells of the eight-cell stage shows that this material is necessary for the complete formation of the embryo, and the results are not due, except in small part, to an indirect effect of the injury. The result is more evident in the case of the two anterior of the upper four cells than in the case of the two posterior. The position of the dorsal lip of the blastopore and of the head on the egg would also lead one to expect that this would be the case.

Comparing the results of this operation with those of Roux in which one of the first two blastomeres is simply stuck with a hot needle, there is a marked difference in the outcome; for, many more of the eggs die or fail to produce an embryo when the upper cells are removed than when the development of one of the first two blastomeres is delayed by the injury. This difference is due mainly to the secondary effects produced by removing the blastomeres, but also, in part, I think, to the effect of removing part of the embryo-forming material. In Roux's operation half of all the kinds of materials are left in the uninjured blastomeres, and bring the uninjured half to its half-development. Furthermore, as Hertwig, Curt Ziegler, Morgan, and Morgan and Torelle have pointed out the injured blastomere in Roux's experiment is, as a rule, only delayed in its development, and is not killed (except rarely). Its »postgeneration« is largely its retarded development.

If, then, the embryo-forming material lies in the upper hemisphere, and is contained in part in the upper four blastomeres of the eight-cell stage, how is this material carried later to or below the equator of the egg? It is my intention to deal later with this question in a separate contribution, which involves a re-examination of the entire early development, but I may anticipate my conclusion here, since it is so intimately bound up with the present question. The movement downwards and outwards of the material of the upper part of the egg is a consequence of the formation and enlargement of the segmentation cavity. This cavity appears at the eight-cell stage, and as it enlarges during the segmentation period its roof becomes thinner, and part of the material that was contained in the upper four blastomeres of the eight-cell stage, and also that contained in the upper ends of the lower four cells, is carried outwards over the roof and sides of the segmentation cavity towards the equatorial regions of the egg. In this region it is in position to take part in the formation of the head and sides of the embryo, forming a ring around the egg which is higher (thicker) in the region of the head.

If the formation of the segmentation cavity is prevented the material of the upper part of the egg remains in position, and the sides of the embryo may develop relatively high up on the egg, as occurs in several forms of abnormal development; such, for example, as result from a centrifugal force or from salt-solutions, or from other agents. When only two of these upper cells are removed the segmentation cavity still appears, especially on the opposite side of the egg, and that half of the embryo develops, not, however, because the segmentation is present, for, the embryo may develop high up on the egg even when the segmentation does not appear, but because the embryo-forming material of that side has not been lost, while the other side, on which the removal occurred, can do no more than form the beginning of its part of the embryo.

By means of this hypothesis which postulates the movement of the materials downwards and outwards we can explain, in large part, the different kinds of abnormal embryos obtained in the experiments that I have carried out on the frog's egg, as well as the result of the experiments here described.

Conclusions.

The appearance of the embryo of the frog on the black hemisphere of the egg in many forms of abnormal development led to the hypothesis, that the material out of which the normal embryo forms, may be contained at first in the upper hemisphere, and become transferred during the cleavage period to the equatorial regions.

In order to determine whether this material is contained, in part, in the upper four blastomeres of the eight-cell stage, these blastomeres were removed by means of a cold needle, or destroyed by a hot needle. In one series of experiments only the two anterior blastomeres (i. e., those on the side of the gray crescent), in other series the two posterior, and in still other series all four upper cells were removed or destroyed.

When the two upper anterior blastomeres of the eight-cell stage are removed defects in the anterior end of the embryo generally occur.

When the two upper posterior blastomeres of the eight-cell stage are removed the anterior end of the embryo generally develops, but the results do not show definitely whether the removal of these cells causes defects in the posterior regions of the embryo, although this appears probable in several cases. This may, however, have been due to indirect effects of the operation. The greater difficulty of identification of the posterior end, and the lower position of the ventral lip (than of the dorsal lip) of the blastopore on the egg renders the results of this operation less determinative than the former.

When all four of the upper blastomeres of the eight-cell stage are removed or destroyed most of the eggs die as the result of the operation; some continue for a while to segment, but do not produce an embryo; a few succeed in forming a circular blastoporic rim (formed by the pulling-in of yolk-bearing cells), and although indications of the presence of ectoderm and of mesoderm above the blastoporic rim occur, there does not seem to be sufficient material present to produce the embryo. The results appear to be due to the removal of embryo-forming material.

Caution must be used in interpreting these results as due only to the removal of embryo-forming material. The most important of these considerations is that more extensive injuries to the egg, than are actually involved in the removal of the blastomeres, can often be shown to take place, so that the injurious effects of the operation may involve neighboring parts of the egg. Another difficulty is found in that different regions of the top of the egg may be included in the upper four cells in different forms of cleavage. A further difficulty arises from the lack of agreement in some cases between the median plane of the embryo and the plane of symmetry of the gray crescent. These, and other difficulties mentioned in the foregoing pages, show that great care must be used in interpreting the results;

nevertheless the results indicate on the whole that some at least of the embryo-forming material that goes towards the formation of the head and sides of the embryo is contained in the upper four blastomeres of the eight-cell stage.

It is suggested that the movement downwards of the material of the black hemisphere that goes to form the embryo is due to the enlargement of the segmentation cavity; as a result much of the more protoplasmic part of the egg is carried to and below the equatorial regions, from which the dorsal portions of the normal embryo derive their material.

July 20, 1904.

Zusammenfassung.

Das Erscheinen des Froschembryo auf der schwarzen Eihemisphäre in einigen Formen abnormer Entwicklung leitete zu der Hypothese, daß das Material, aus welchem sich der normale Embryo bildet, zu Beginn in der oberen Hemisphäre enthalten sein kann und während der Furchungsperiode nach der Äquatorialregion geschoben wird.

Um zu bestimmen, ob dies Material zu einem Teil in den oberen vier Blastomeren des Achtzellenstadiums enthalten ist, wurden diese Blastomeren mittels einer kalten Nadel entfernt oder mit einer heißen Nadel zerstört. In einer Versuchsreihe wurden die beiden vorderen Blastomeren (d. h. diejenigen auf der Seite des grauen Halbmondes Rouxs), in andern Versuchsreihen die beiden hinteren und in noch andern Versuchsreihen alle vier oberen Zellen entfernt oder zerstört.

Wenn die beiden vorderen oberen Blastomeren des Achtzellenstadiums entfernt werden, entstehen im allgemeinen Defekte im vorderen Ende des Embryo.

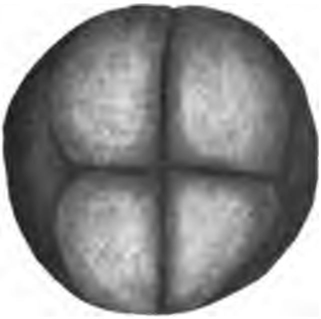
Wenn die beiden hinteren oberen Blastomeren des Achtzellenstadiums entfernt sind, so 'entwickelt sich im allgemeinen das vordere Ende des Embryo. aber die Resultate zeigen nicht definitiv, ob der Ausfall dieser Zellen Defekte in den hinteren Regionen des Embryo erzeugt, obgleich dies in einigen Fällen wahrscheinlich erscheint. Dies kann jedoch indirekten Effekten der Operation zuzuschreiben sein. Die größere Schwierigkeit, das hintere Ende wiederzuerkennen, und der Umstand, daß die ventrale Blastoporuslippe am Ei tiefer liegt als die dorsale, machen die Resultate dieser Operation weniger bestimmt als die ersteren.

Wenn alle vier oberen Blastomeren des Achtzellenstadiums entfernt oder zerstört werden, so sterben die meisten Eier infolge der Operation; einige fahren noch eine Weile fort sich zu teilen, bringen aber keinen Embryo zustande; einige wenige erreichen es, einen kreisförmigen Blastoporusrand zu bilden (gebildet durch die Einstülpung der Dotterzellen), und obgleich Andeutungen von Ektoderm und von Mesoderm über dem Blastoporusrand vorhanden sind, so scheint sich hier nicht genügend Material zu finden, um einen Embryo zu bilden.

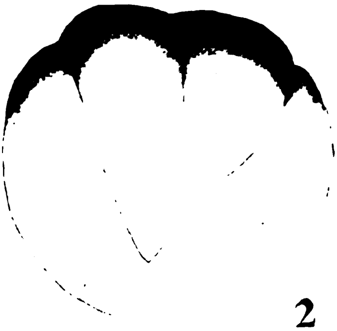
Die Resultate müssen, wie es scheint, der Entfernung des embryobildenden Materials zugeschrieben werden.

Man muß vorsichtig sein bei der Deutung dieser Resultate als Folgeerscheinung der Entfernung des embryobildenden Materials allein. Das Wichtigste dieser Betrachtungen ist, daß offenbar ausgedehntere Verletzungen des Eies, welche in Wirklichkeit die Entfernung der Blastomeren mit sich bringt, Platz greifen, so daß die schädlichen Folgen der Operation Nachbarteile in dem Ei ergreifen können. Eine weitere Schwierigkeit liegt darin, daß verschiedene Regionen der Oberseite des Eies in den vier oberen Zellen bei verschiedenen Furchungsformen enthalten sein können. Eine weitere Schwierigkeit rührt in einigen Fällen von der fehlenden Übereinstimmung zwischen der Medianebene des Embryo und der Symmetrieebene des grauen Halbmondes her. Diese und andre Schwierigkeiten wurden auf den vorhergehenden Seiten erwähnt; sie zeigen, daß man große Vorsicht bei der Deutung der Resultate anwenden muß; nichtsdestoweniger zeigen die Resultate im ganzen, daß wenigstens einiges von dem embryobildenden Material, welches bei der Bildung des Kopfes und der Seiten des Embryo beteiligt ist, in den oberen vier Zellen des Achtzellenstadiums enthalten ist.

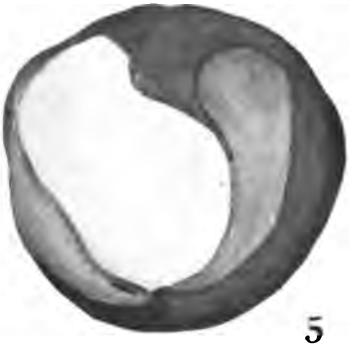
Es wurde angedeutet, daß die Abwärtsbewegung des Materials der schwarzen Hemisphäre, welches bei der Bildung des Embryo beteiligt ist, der Vergrößerung der Furchungshöhle zuzuschreiben ist; ein Resultat dieser Bewegung ist, daß viel von dem mehr protoplasmatischen Teile des Eies zu den und unter die äquatorialen Regionen, aus welchen die dorsalen Teile des normalen Embryo ihr Material beziehen, befördert wird.



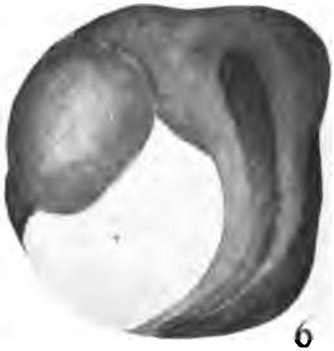
1



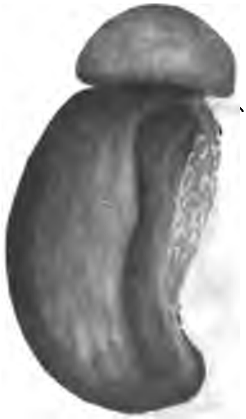
2



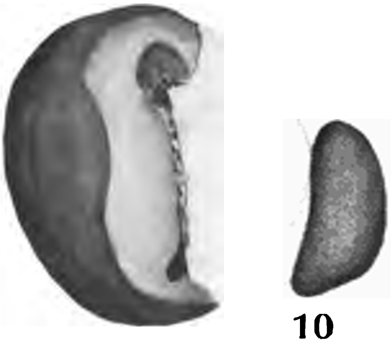
5



6

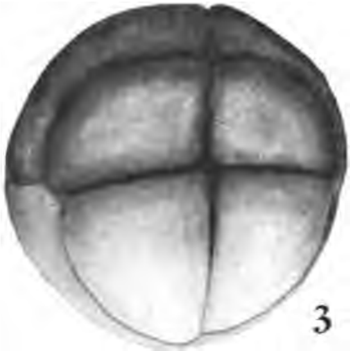


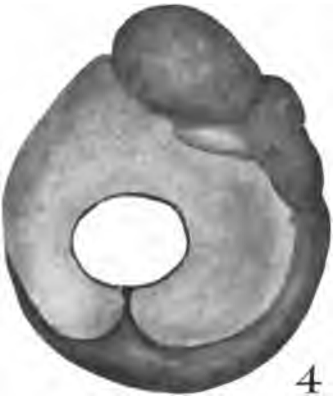
9

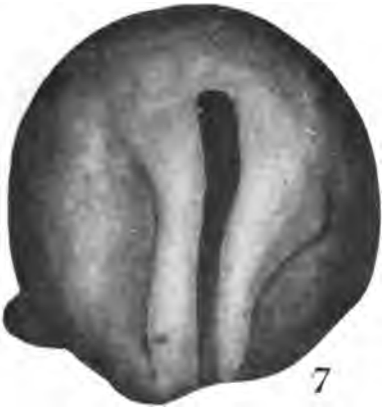


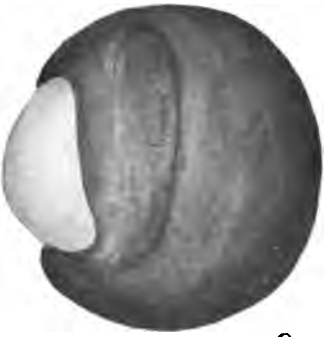
10

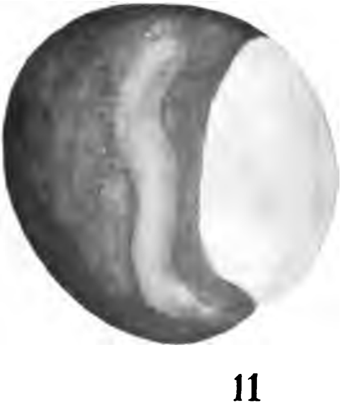
Verlag von Wilhelm

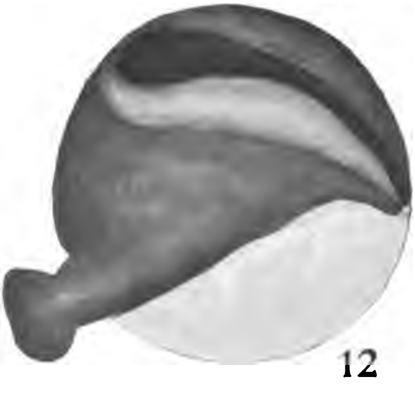

3


4


7

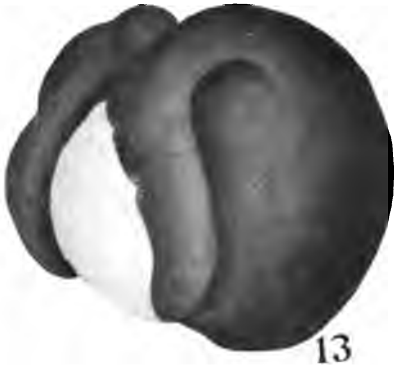

8

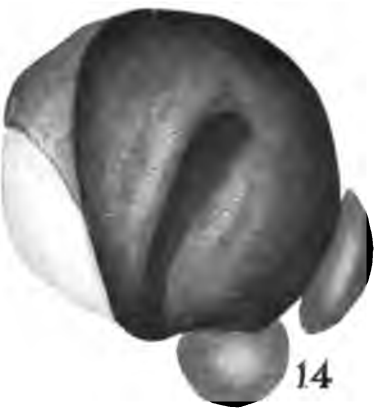

11

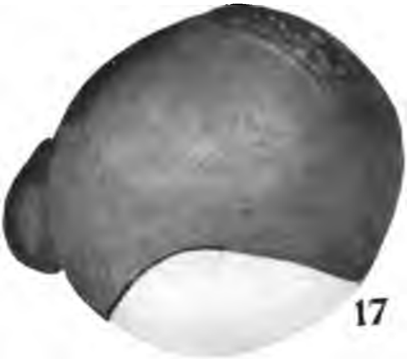

12

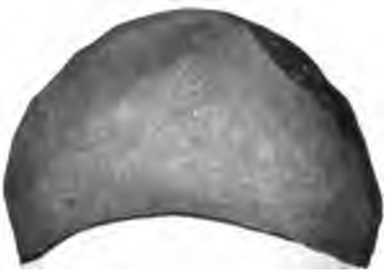
UofM

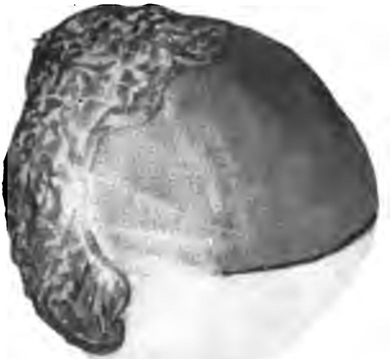
Engelmann in Leipzig.

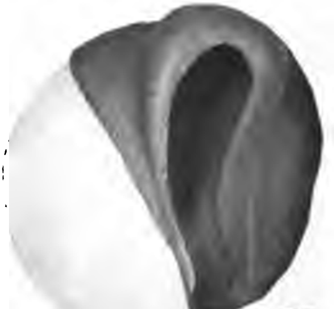

13


14

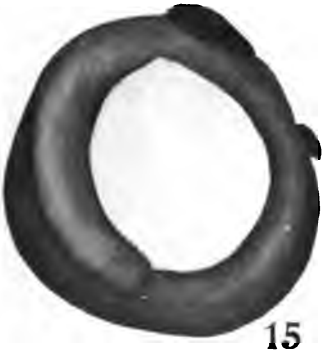

17


18

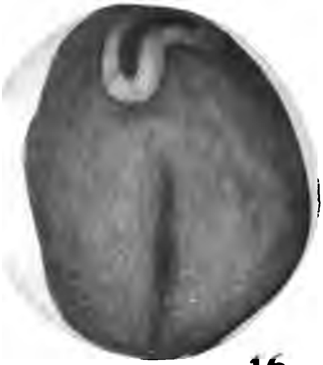

21


22

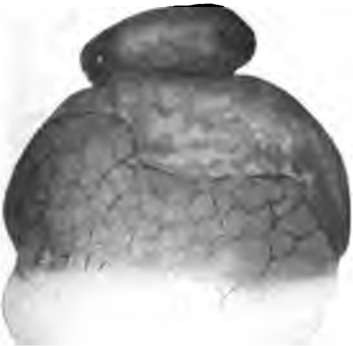
Verlag von Wilheln



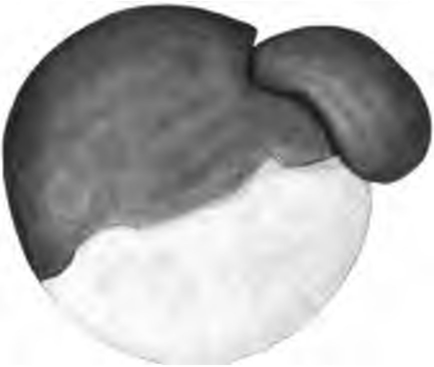
15



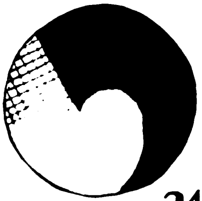
16



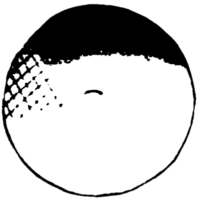
19



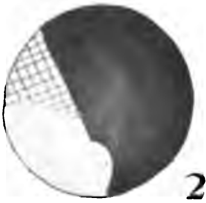
20



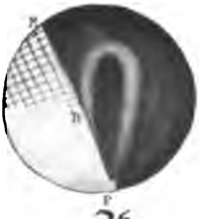
24



23



25



26

UofM

Engelmann in Leipzig.

Experimental Studies on the Eye of the Frog Embryo.

By

Helen Dean King.

With Plate VI.

Eingegangen am 21. November 1904.

It was shown by BONNET (3) in 1780, that if the greater portion of the eye of *Triton* is removed and only a small piece remains attached to the optic nerve, a new eye will regenerate in the course of a few months. This result was confirmed later by the experiments of BLUMENBACH (2), PHILIPPEAUX (13), and COLUCCI (4). At the present time, interest in experimental work on the eye of amphibians seems to be concerned principally with the origin and manner of formation of a new lens after complete or partial extirpation. Much careful work has already been done along this line, noticeably by WOLFF (20—22) and by FISCHEL (5—7) both of whom have worked on the lens formation in the eye of *Triton* which seems to be a very favorable subject for such investigations.

The experiments recorded in the present paper were begun in the spring of 1900; but before any definite conclusions had been reached, SPEMANN (18) published the results of a small series of experiments on young embryos of *Rana fusca.* In these experiments SPEMANN destroyed one side of the head before the medullary folds had closed in order to ascertain whether the various processes in the development of the eye are correlated or not. From his results, SPEMANN concludes that an optic vesicle can change into an optic cup when the influence of the lens is barred out, and that the formation of a lens is dependent on contact between the epidermis and the optic cup: the optic cup, it appears, must reach a definite grade of development before a lens arises, and no lens forms before this time. The first of SPEMANN's conclusions, i. e. that the optic vesicle can change into an optic cup without the presence of a lens, I can fully confirm; but my results do not support SPEMANN's other conclusions as will be shown later.

I. Method.

Embryos of *Rana palustris* were used for all experiments, and, with the exception of those described in Section II, they were operated upon at the stage of development when the brain vesicles are well defined and the medullary folds are beginning to unite. In making the operation, the embryo was transferred to a piece of moist filter paper placed on the stage of a dissecting microscope, and then a puncture was made with a heated needle on one side of the head in the region of the fore-brain from which the optic vesicle would normally develop.

The operation is a severe one, and about one-half of the 800 tadpoles operated upon died within twenty-four hours as a direct result of the injury. Of the embryos that survived the operation, nearly one-fourth, when killed and sectioned, showed perfectly normal eyes on both sides of the head; in the remaining embryos either an eye was lacking on the injured side of the head or, if one formed, it was more or less abnormal in shape and position. This lack of uniformity in the results is due, in part at least, to the fact that it is impossible to heat the needle at uniformly the same temperature in all of the operations, and also to the fact that the puncture did not always injure exactly the same region of the fore-brain.

Removing the entire eye from an embryo in which the lens and cornea have already formed is a comparatively simple operation and one that is rarely followed by fatal results if sufficient care is taken during the operation not to injure the surrounding tissues. If a heated needle is inserted into the centre of the optic cup, the tissues adhere to its surface and the entire eye can be withdrawn at once. The tadpole very soon recovers from the shock of the operation, and the injured surface is entirely healed over in the course of two or three days.

II. The Effects of a Total Extirpation of the Eye of a Tadpole After the Formation of the Lens and the Cornea.

Experiments made by Philippeaux and by Colucci have shown conclusively that the adults of various species of amphibians are not able to regenerate an eye after the total extirpation of the old one. Amphibian embryos, however, have a much greater power of regeneration than have the adults, and it might be possible that the embryonic tissues of the head would possess the power to regenerate a new eye, although this power is totally lacking at a later period of development. To test this point the eye was removed from a number of tadpoles measuring about 12 mm. in length. Only seven out of total of 125 tadpoles that were operated upon died in the course of the week following the operation, and in these cases the brain had been injured when the eye was removed, as one side of the head was greatly distorted after the wound had healed. Beginning about a month after the operation and continuing for a number of weeks, a few of the embryos were killed at intervals of five days, although there was no external evidence of an eye on the injured side of the head in any case. Serial sections through the heads of these embryos failed to show, in a single instance, the slightest indication of a regeneration of the missing eye. The outer ectoderm, which had completely covered the injured region in the course of 2—3 days after the operation, appeared to be of the same thickness and the cells seemed to have the same general character as those on the uninjured side of the body. The region of the head from which the eye had been removed was always found to be filled with loose mesenchyme tissue. Occasionally a small amount of pigment was present. This pigment had either been introduced from the outer ectoderm when the operation was made, or, possibly, it was a part of the pigmented coat of the eye that had remained behind when the eye was removed. In most of these embryos the tissues of the head, even those which had been in close contact with the extirpated eye, showed not the slightest trace of the operation, although in a few cases the forebrain appeared somewhat distorted and its wall was thinner than normal on the injured side.

The results of this series of experiments on amphibian embryos are in accord with those obtained by other investigators on adult forms: in neither case is it possible to obtain the regeneration of an eye after total extirpation. As a complete optic cup and a lens can regenerate from a very small fragment of the eye, it is evident that the eye tissues are able to produce any part of the eye that may be lacking: the other tissues of the head, however, do not possess this power which is as completely lacking in the embryo as in the adult.

III. The Effects of Totally Destroying the Eye-forming Region of the Fore-brain before the Closure of the Medullary Folds.

If the portion of the fore-brain that normally produces an optic vesicle is totally destroyed before the medullary folds have closed, have the other parts of the brain or any other tissues of the head the power to produce an eye and thus restore the normal form of the embryo? This was one of the problems I had in mind when these experiments were begun.

Many of the young embryos in which the fore-brain was punctured before the closure of the medullary folds showed no traces of an eye on the injured side of the head, although, in some cases, they were kept alive for three weeks after the operation. In every instance there was a well developed optic cup on the uninjured side of the head, and a lens had formed or was in the process of formation from the ectoderm. In nearly all of these embryos the wall of the fore-brain was much thinner than normal on the injured side, in some instances being only one or two rows of cells in thickness. The ectoderm covering the wounded surface seemed perfectly normal in all cases and the region in which the eye would normally have developed was filled with a large number of mesenchyme cells. Usually some scattered pigment granules were also present as was found in the experiments in which an entire eye was removed from an older embryo (Section II).

It is evident that, in these cases, all of the eye-forming tissue on one side of the head was either destroyed by the operation or rendered incapable of development and that no other tissue of the head has the power to produce an eye.

IV. The Development of a Normal Eye After a Puncture of the Fore-brain.

A number of embryos operated upon before the closure of the medullary folds developed perfectly normal eyes on both sides of the head. In some cases the effects of the operation were to be seen in an unusual thinness of the wall of the fore-brain; in other cases the brain no longer occupied its normal position in the median plane of the body but was drawn over towards the injured side, doubtless as a result of the way in which the wounded surface healed.

It is probable that, in these embryos, the region of the brain affected by the operation lay in front of or at the side of the eye-forming region; therefore, as the eye-forming tissue was not injured, the development of the eye could proceed in a perfectly normal manner in spite of the injury. In all of these embryos the eyes maintained their normal relations with respect to the surrounding tissues, and both eyes of an embryo were usually in the same stage of development, although in some few instances the lens on the uninjured side of the body was in a slightly later stage of development than was the one on the side that had been punctured.

V. The Formation of an Optic Cup without the Formation of a Lens, or without any Connection between the Eye-forming Tissue and the Brain.

If only a portion of the brain tissue that normally forms the optic vesicle is destroyed by the heated needle, the remaining tissue continues its development, somewhat slowly, and eventually becomes a more or less normal optic cup regardless of its location or of its connection with the rest of the brain. Fig. 1 shows a very extreme case of this kind. The tadpole from which this drawing was made was operated upon the second of April, 1902, and was killed four days later. Three days after the operation this embryo attracted attention because it was the only one of the 27 embryos in the series that did not show an eye on either side of the head. It was somewhat less developed than the other tadpoles in the series at this time and it swam very feebly and in an irregular manner, resting on one side when it reached the bottom of the dish in which it was kept. A complete series of sections through the head of this embryo shows the presence of but a single eye (Fig. 1, *O.C.*) which was not visible through the deeply pigmented ectoderm of the body wall because it occupies a very abnormal position between the floor of the fore-brain (Fig. 1, *F.B.*) and the roof of the pharynx (Fig. 1, *Ph.*). This eye, which is fairly normal in shape, is in the optic cup stage of development, and it measures 0.3 mm. in diameter through the line *XY*, thus being approximately the same size as the normal eyes found in other embryos of the same age. No lens is present in any section of the eye of this embryo, and in no place is there a thickening of the inner layer of ectoderm that might possibly be considered an early stage in the formation of a lens. The eye is entirely surrounded by mesodermal tissue and there is no trace of an optic nerve. It is evident, therefore, that this eye has developed through the stage of the optic vesicle to that of the optic cup without connection with the brain and without the presence of a lens.

The very abnormal position of this optic cup can doubtless be attributed to the fact that in the operation a portion, or perhaps all, of the eye-forming tissue of the fore-brain was separated from the rest of the brain and yet not greatly injured. In the process of healing, this eye-forming tissue came to occupy a position between the brain and the pharynx and here it continued to develop as long as the embryo lived. It is obvious that an eye in this location can be of absolutely no use to the organism, no matter if is should continue it development and should become an entirely normal structure. The formation of such an eye seems inexplicable unless we believe with SPEMANN (18), »daß in der Medullarplatte die einzelnen Teile des Augenbechers schon bestimmt sind«, and, therefore, that the portion of the fore-brain that normally forms the eye is so specialized that it will develop into an eye so matter what the surrounding conditions may be. Apparently the power of self-differentiation must be conceded to eye-forming tissue as it is capable, under certain conditions, of developing into a fairly normal structure when entirely removed from its normal relations with the other structures of the head (Fig. 1).

Fig. 2 shows an outline drawing of a section through the eye region of an embryo killed four days after the puncture of the left side of the fore-brain. The brain itself shows no signs of the operation, and on the right side of the head there is a normal eye with a well developed lens. On the left of the fore-brain is an optic cup, absolutely normal in shape and in the structure of its cells, yet its greatest diameter through the line *AB* is but 0.20 mm., while that of the normal eye through the line *CD* is 0.35 mm. There is no sign of a lens in connection with this small optic cup, nor is there any thickening of the ectoderm in the vicinity of the eye that might be considered to be an early stage in the formation of a lens. This embryo shows the power of regulation possessed by the organism, as the portion of the eye-forming region of the fore-brain remaining after the operation did not produce a fragment of an optic cup of usual size but developed into a very small optic cup of normal shape. I can see no reason why this eye could not have become functional had a lens developed later in connection with it.

A portion of a section through the head region of an embryo operated upon the nineteenth of April, 1903, and killed six days later is shown in Fig. 3. On the left side of the head, which was uninjured, there is a normal eye with a lens already completely separated from the ectoderm. The right eye, which is shown in Fig. 3, comes directly from the fore-brain whose wall on this side is formed of only one to two layers of small cubical cells. This eye has presumably reached the stage of the optic cup although its shape is very unlike that of a normal eye at the same period of development. The upper edge of the optic cup is in direct contact with the inner layer of ectoderm; but in no section of the head is there any indication of a thickening of the ectoderm to form a lens. If, as maintained by some investigators, the formation of a lens is dependent on contact between the optic cup and the ectoderm, it is not clear why a lens has failed to form in this case as the normal eye on the opposite side of the head developed a lens at least three days before the embryo was killed. It is, of course, obvious that the right eye in its present condition is so distorted that a lens would be useless even if it had developed; but, as will be shown later, many eyes almost, if not quite, as abnormal as this one have a well developed lens and, therefore, the abnormality of the eye alone should not have prevented the formation of a lens. It may be possible that the embryo was killed before the eye on the injured side had reached the proper stage of development for it to be able to cause the formation of a lens from the ectoderm; but it is, of course, impossible to determine this point definitely.

In five other embryos I have found a well developed optic cup on the injured side of the head that had no connection whatever with any part of the brain. The conditions in these embryos, however, were not as abnormal as were those in the embryo shown in Fig. 1, as in each case there was a perfectly normal eye on the uninjured side of the head, and the eye that had formed on the punctured side, although it was entirely separated from the brain, was in its normal position in the head and had the opening of the cup turned towards the exterior of the body. In three embryos a lens had developed from the ectoderm and occupied its usual position in the eye; in the other two embryos no lens had developed on the injured side when the embryos were killed. The development of the eye on the injured side of the head always lagged behind that of the normal eye, although apparently taking place in the usual manner.

In one of his experiment on *Hyla viridis* SCHAPER (15) cut off the anterior part of the head of an embryo in such a way that the right eye was entirely removed while the left one remained in its normal position. When this embryo was killed, eleven days after the operation, it was found that the fore-brain was entirely lacking and that there had been no regeneration of the missing eye. The left eye was somewhat smaller than the eye of a normal embryo at the same stage of development, although it appeared in every other way to be perfectly normal, and it had a well developed lens. The interesting fact about this eye is that it had no optic nerve and, consequently, it had absolutely no connection with the portion of the brain remaining after the operation. In discussing the result of this experiment, SCHAPER states: »Wir können nicht umhin anzunehmen, dass während der vorliegenden Entwickelungsperiode funktionelle Reize auf Wachsthum und Differenzirung des erhaltenen Auges keinerlei Einfluss ausgeübt haben: seine Weiterentwickelung geschah lediglich nach den Principien der Selbstdifferenzirung.« This principle of »self-differentiation« is the only one apparently, that is adequate to explain the development of an optic cup in such cases as the one described by SCHAPER and in those of my own experiments in which an optic cup developed without any connection with the brain.

The results of the experiments in this series confirm the conclusion of SPEMANN and of RABL (14) that an optic cup can develop from an optic vesicle when there is no lens present. To this conclusion may, perhaps, be added that the change from an optic vesicle into an optic cup is likewise independent of any connection with the brain and that it is the direct result of a process of self-differentiation in the eye-forming tissue of the fore-brain.

VI. The Formation of a Lens from the Ectoderm in Connection with the Development of an Optic Cup.

An eye that develops in an embryo of *Rana palustris* after a puncture of the eye-forming region of the fore-brain usually possesses a lens which is more or less normal in structure. As presumably the operation destroyed that portion of the ectoderm that would normally produce a lens, the question arises as to whether there is a definite region of the ectoderm that alone is capable of producing a lens, or whether any portion of the ectoderm of the head has the power to form a lens provided it receives the proper stimulus. It is possible also that, under certain conditions, a lens can form from the edge of the optic cup, as in *Triton*, and thus enable an eye which can not obtain a lens from the ectoderm to become functional when it has reached the proper stage of development.

In making the operations for this series of experiments, the punctures were intentionally made in different regions of the forebrain in order to produce as many kinds of abnormal eyes as possible. This was done in the hope that it might be possible to determine the extent of the power possessed by the optic cup as well as by the ectoderm of the different regions of the head to form a lens.

After operations of this character in which it is impossible to determine the extent of the injury until the embryos are killed and sectioned, the results are rarely found to be uniform and it is not possible to draw any general conclusions from a small number of experiments. The cases that are described in this sections were selected from over 150 embryos in which the eye on the injured side of the head was more or less abnormal. That so few of the embryos gave results from which conclusions could be drawn can be attributed either to the fact that a lens had not yet developed in connection with the eye on the injured side of the head when the tadpole was killed (although it had always formed in a perfectly normal manner on the uninjured side); or to the fact that a lens had already developed so far when the embryo was killed that it was not possible to determine its origin. The chances of being able to determine the origin of the lens on the injured side of the head are most favorable if the embryo is killed from three to four days after the operation.

Fig. 4 shows a portion of a section through the head of an embryo that was operated upon the thirty-first of March 1903, and killed four days later. The embryo, therefore, lived two days less than the one from which Fig. 3 was drawn and presumably its organs were correspondingly less developed. Sections through the head of the embryo showed, on the uninjured side, a normal eye with its lens entirely separated from the ectoderm; on the injured side of the head, as shown in Fig. 4, there projects from the base of the fore-brain a very abnormal optic cup. The inner layer of this eye is a nearly straight mass of cubical cells which curves but slightly at its upper edge where it joins the outer pigmented layer. Although this optic cup is fully as abnormal as the one shown in Fig. 3, yet it has a well formed lens (Fig. 4, *L*) entirely separated from the ectoderm and in contact with the upper edge of the cup. The lens, which was probably derived from the ectoderm in a normal manner, is very much smaller than the one on the opposite side of the head as it measures but 0.046 mm in diameter, while the lens of the normal eye measures 0.107 mm. in diameter. In this embryo it is not probable that the edge of the abnormal optic cup ever came in contact with the ectoderm, and yet a lens has developed from the ectoderm although the conditions for its formation were apparently not as favorable as they were in the case of the eye shown in Fig. 3.

An outline drawing of a section through the eye region of an embryo killed five days after the puncture of the fore-brain is shown in Fig. 5. On the right side of the head is a normal eye with its lens completely separated from the ectoderm. On the left side of the head, the fore-brain is connected through an optic stalk with a very abnormal eye whose structure in greater detail is shown in Fig. 6. This eye is exceedingly small, its diameter through the line *AB*, Fig. 5, being 0.116 mm., while that of the normal eye through the line *CD*, Fig. 5, is 0.334 mm. The outer edge of the optic cup is 0.071 mm. away from the inner layer of ectoderm, yet there projects from the latter, opposite the opening of the cup, a structure that unquestionably must be considered as an early stage in the formation of a lens. Instead of developing in a normal way, this lens, which measures 0,025 mm. through the line *XY*, Fig. 6, is a somewhat rounded mass of cubical cells attached by a thick stalk of similar cells to the inner layer of ectoderm, the whole structure extending 0,088 mm. from the ectoderm. Another section of this eye shows the lens lying very close to the upper edge of the optic cup and containing a central cavity. As the embryo was killed before the lens had broken away from its attachment to the ectoderm there can be no question of it its origin and manner of formation.

In all cases in which it is possible to determine definitely the origin of the lens found in connection with an abnormal eye, it is certain that the lens is derived from a thickening of the ectoderm. Very frequently the abnormal eye lies some distance behind the plane of the normal eye on the opposite side of the head and yet the ectoderm of this region of the head is as able to produce a lens as is that more anterior. In some cases, as in Fig. 9, a lens may form from the ectoderm near the upper edge of the optic cup; in other cases the lens may develop near the lower edge of the cup (Fig. 7); in still other embryos (Fig. 8) there may be a thickening of a considerable portion of the ectoderm in the vicinity of the optic cup. In the eye show in Fig. 8, the thickening for a lens occupies all of the region in front of the opening of the optic cup for several sections, and as the eye is much further back in the head than is the normal eye on the uninjured side, the ectoderm in front of it, could not possibly be that which would normally produce the lens.

The recent experiments of Lewis (10) have shown conclusively that not only is the possibility of lens formation present in all ot the ectoderm of the head, but also that the ectoderm from other portions of the body, if grafted over an optic cup, will develop into a lens. The power to form a lens must, therefore, be conceded to all of the body ectoderm, and is not to be limited to the descendents of the cells of the sense plate as believed by Schaper (16).

It is stated by Rabl that »die Ursache für die Entstehung der Linse in einem formativen Reize zu suchen, der von dem freien Ende der Augenblase auf die Berührungsstelle mit dem Ektoderm ausgeübt wird«. This opinion is also held by Spemann (18) and by Lewis. The latter investigator states that »epithelial cells which normally give rise to a lens do not do so when the optic vesicle fails to come in contact with them for a sufficient length of time or at the proper stage«. From the results obtained in some of the above experiments it seems to me certain that contact between the optic cup and the ectoderm is not the only stimulus that is capable of causing the development of a lens. In the case of the eye shown in Fig. 6, the edge of the optic cup could never have come in contact with the ectoderm, and yet a lens is forming, unquestionably, from a thickening of the inner ectoderm opposite the eye. In some of his experiments, Lewis also obtained a lens from the ectoderm when the optic cup was imbedded some distance in the head and in such cases the lens always developed on a pedicle in a way similar to that shown in Fig. 6. Lewis explains these cases as follows: »A long epithelial process was probably formed at the time of the healing of the operation wound and thus gave the deeply placed eye a chance for contact with the ectoderm«. It seems to me rather remarkable that this long epithelial process, purely an accidental result of the operation, should invariably be formed directly opposite the opening of optic cup, and that it is never found in cases in which the optic cup is close to or in contact with the ectoderm. Such an explanation does not seem to me adequate in the case shown in Fig. 6, or in others of the same nature which I have obtained. In my experiments the character of the operation was such that the portion of the ectoderm in contact with the heated needle was usually destroyed and, even if it was shoved inside the head, it would have been rendered incapable of development. In all of the hundreds of tadpoles that I have sectioned after such operations I have never found a similar projection of ectoderm in the injured region of the head unless it was directly opposite an optic cup and was forming a lens. In some cases the pedicle of the lens was long, in others short, depending entirely on the distance between the optic cup and the ectoderm. Contact between the optic cup and the ectoderm, on the other hand, does not necessarily lead to the formation of a lens. In the case of the eye shown in Fig. 3, the upper edge of the optic cup is in direct contact with the inner ectoderm and yet there is no sign of lens although one has developed on the opposite side of the head. In some instances Lewis failed to obtain a lens when the optic cup was in contact with the ectoderm, and he considers this to be due either to the fact that the optic cup had not been in contact with the ectoderm for a sufficiently long period of time or that it had not reached the proper stage of development for it to be able to cause the formation of a lens. Contact between the optic cup and the ectoderm may, in some cases, furnish the stimulus that calls forth the formation of a lens; but that it is not the only stimulus that can produce this result is evident from such cases as that shown in Fig. 6. Whether the stimulus for lens production always comes from the optic cup, when one it present, or whether it may come from the nervous system or from some other source I have not been able to determine.

VII. The Formation of a Lens-like Structure from the Ectoderm without the Presence of an Optic Cup.

That a lens-like structure can develop from the inner ectoderm when an optic cup is totally lacking on the injured side of the head is shown by the two following cases selected from seven of the same general character that were obtained during the course of these experiments. Part of a section through the head of one of these embryos is shown in Fig. 9. On the left side of the head, which was uninjured, the eye is somewhat abnormal, and it has a lens which has not yet separated completely from the ectoderm. On the right side of the head there is absolutely no sign of an optic cup, and the wall of the fore-brain is much thinner than normal, thus showing the severity of the operation. On the injured side, directly opposite the place where the lens is forming for the normal eye, there is a pronounced thickening of the inner ectoderm (Fig. 9, *L*). This thickening of the ectoderm, which is shown more highly magnified in Fig. 10, extends over four sections of the head and is like the lens forming on the opposite side of the head except that it lacks a central cavity and is somewhat smaller, measuring but 0.034 mm. in diameter while the lens on the opposite side of the head measures 0.053 mm. in diameter.

Another case in which a lens-like structure formed without the presence of the optic cup is shown in Fig. 11. In this instance the structure is somewhat larger than that shown in Fig. 10, as it measures 0.075 mm. in diameter through the line *X Y* Fig. 11, and is precisely similar in structure to the lens for the normal eye of an embryo at the same stage of development. In this case, also, an optic cup was entirely lacking on the injured side of the head, although a perfectly normal eye was present on the opposite side of the head and its lens was still connected with the ectoderm, being but a little more developed than the one shown in Fig. 9.

The other cases I have found in which an optic cup was lacking and yet a lens-like structure was developing from the ectoderm are very similar to those shown in Figs. 9—11. Unfortunately, I have no older stages that show whether, under these abnormal conditions, this structure would continue to develop and eventually separate from the ectoderm.

These results apparently furnish evidence in favor of the view that in a definite region of the ectoderm the cells are destined to form a lens and, therefore, that they will produce such a structure even if an optic cup is lacking. That a lens should develop under such abnormal conditions is not more surprising than is the fact that a portion of the eye-forming region of the fore-brain will continue its development and form a fairly normal optic cup (as shown in Fig. 1) even when it is removed from all of its normal relations, cut off entirely from all connection with the brain, and surrounded by mesenchyme tissue. It seems evident that the power of self-differentiation is possessed in a remarkable degree by those tissues in the head of the frog embryo which normally form the optic cup and the lens; and each of these structures can apparently develop, to a certain extent at least, absolutely independently of the other. The change of an optic vesicle into an optic cup and of the ectodermal cells into a lens, is due, apparently, not as much to correlative differentiation as to unknown causes inherent in the cells themselves.

The formation of a lens from the ectoderm without the presence of an optic cup has, as far as I am aware, been found as yet by but one investigator, MENCL (11), who has described such a case in a very abnormal *Salmo salar* with two heads. One head appeared perfectly normal; but the other showed no eyes externally. When the latter head was sectioned, there was found absolutely no sign of an optic cup on either side of the head, although there were lenses on both sides, the left one being more dorsal and situated somewhat further back than the one on the right. Both lenses were fairly normal in structure, although the left one was somewhat smaller than the right and was still connected with the ectoderm from which it originated without question. From this case MENCL states that we must admit that the lens of the eye can arise independently of the formation of the optic cup, and he adds further: »Die Hirnaugen mit epidermalen Linsen treten bei den Wirbelthieren als eine neue, ausschließlich ihnen gehörende Erwerbung auf, und sie bilden sich im Bereiche eines bestimmten Kopfsegmentes; die ganze diesem Segmente zugetheilte Epidermis ist Träger einer gewissen Tendenz, die darin besteht, im Verlaufe einer gewissen Entwickelungsstufe die Linse zu bilden. Wenn auch die Augenblasenbildung völlig ausbleibt, was in einzelnen anomalen Fällen zu Stande kommt, so werden doch die Augenlinsen, obzwar zwecklos, gebildet. Der diese zwecklose, wie durch Erinnerung der Epidermiszellen auftauchende Linsenbildung auslösende Faktor ist die Vererbung.«

In the figures given in MENCL's paper showing the location of the two lenses, there is very little space between the brain and the body ectoderm, and on one side the lens lies in a well defined depression of the brain wall. SPEMANN (19) has offered a very different explanation of the formation of these lenses from that given by MENCL. He believes that there is only an apparent lack of the optic cups, the wall of the brain near the lenses in his estimation »nichts anderes ist, als die nicht abgegliederte und außerdem nachträglich rückgebildete Retina«. There is absolutely nothing in the structure of the brain wall in the figures shown by MENCL that would indicate that it is a degenerate retina. If we accept SPEMANN's explanation, then MENCL's idea of the self-differentiation of the lens is, of course, valueless.

The results of the experiments on embryos of *Rana palustris* in which a lens-like structure developed without the presence of an optic cup appear to support MENCL's position. In the case shown in Fig. 9, the brain wall on the injured side, which is much thinner than normal, is 0.233 mm. away from the ectoderm in the region where the lens-like structure has formed, and all of the cells of which it is composed are apparently similar in structure. There is not the slightest reason, as far as I can see, for assuming that the wall of the brain opposite the place where this structure is forming represents the retina, and, even if it did, it is so far from the ectoderm that it would seem that it could have no influence whatever on the lens formation. It appears that the power of self-differentiation must be granted to lens-forming cells of the ectoderm in the embryo of *Rana palustris*, and it seem probable that a stimulus similar to that which is capable of causing the formation of lens-like structures in the ectoderm of the frog embryo when an optic cup its lacking might also have acted to produce the lens-like structures in the *Salmo salar* described by MENCL.

VIII. The Formation of a Lens from the Edge of the Optic Cup.

In all of the embryos so far described, and in a large number of others where abnormal eyes were formed, the lens, if it developed at all, was unquestionably derived from a thickening of the inner layer of ectoderm as is the case in normal development. In *Triton* and in others of the Urodela where the lens has been extirpated from the eye of a young or of an adult animal, a new lens arises in the course of a few weeks or months, not from the ectoderm, but from the iris. The manner of its formation in *Triton* according to Wolff (20) is as follows: »Das innere epitheliale Blatt der Iris verliert sein Pigment, welches von massenhaft herbeieilenden Leukocyten fortgetragen wird, am Pupillarrande wuchert das Epithel, aus diesen Wucherungen entsteht am oberen Rand der Pupille ein Linsensäckchen, und aus diesem Linsensäckchen bildet sich — nunmehr nach vererbtem Typus — die vollständig normale Tritonenlinse.« It appears also, from the recent experiments of Barfurth and Dragendorff (1) that a new lens can be formed from the edge of the optic cup in the young chick embryo, although the power to produce a lens seems to be entirely lacking in the eye of the adult bird.

The experiments of Müller (12) and also those of Fischel (5—7) have confirmed the earlier work of Colucci and of Wolff regarding the origin of the lens from the edge of the iris in the eye of *Triton*. Fischel has also shown that if the iris is intentionally injured in several places more than one lens may be formed; and that not only any part of the iris is capable of forming a lens, but also that lens-like structures can be produced from the retina.

The eye of the Urodela is thus found to be capable of regenerating a lost lens, but instead of being produced from a thickening of the ectoderm as one might expect would be the case from the course of the normal development of the lens in ontogeny, the new lens arises from the iris of the eye and generally from its upper edge. The reason for the formation of the lens at the upper edge of the iris was first considered to be due to the action of gravity on the eye, but an ingenious experiment made by Wolff (21) has shown that the new lens regenerates from the upper edge of the iris even when the animal is kept constantly upon its back and therefore gravity does not determine it location. According to Wolff it is »nicht das Fehlen, sondern der Verlust der Linse, nicht die einzige auslösende Ursache, sondern eine wesentliche Vorbedingung für die Regeneration zu bilden scheint«. Fischel, on the other hand, believes that »die auslösende Ursache liegt vielmehr stets in der durch die Operation bewirkten Reizung der Iris«.

In explanation of the formation of the new lens in the eye of *Triton* from the iris rather than from the ectoderm, Schimkewitsch (17) has stated that: »Man darf nicht vergessen, dass die Linse der paarigen Augen eine spätere Bildung ist, und dass es sehr wahrscheinlich ist, dass ursprünglich die paarigen Augen eine ebensolche Linse besaßen, wie wir sie im unpaaren Auge der *Hatteria* sehen,

d. h. hervorgegangen aus der Wand der Augenblase selbst.« The formation of the lens from the edge of the iris, on this assumption, is merely a return to ancestral conditions and might, if true, very possibly be met with occasionally in the course of the normal development of an individual.

In his most recent paper, FISCHEL (7) has stated that the formation of a lens from the edge of the iris is easily explained if one but assume, »dass die Fähigkeit zur Linsenbildung nicht allein einem ganz bestimmten Gebiete des Ektoderms, sondern ursprünglich allen (mindestens in einem bestimmten Körperabschnitt) Zellen dieses Keimblattes zukommt. Es ist klar, dass diese Annahme auch durch die hier mitgetheilten Thatsachen eine gewisse Stütze erhält: entstammen doch die Zellen der Iris und Retina, deren Fähigkeit zur Linsenfaserdifferenzirung erwiesen wurde, gleichfalls dem Ektoderm. Fasst man diese Annahme in die Form, dass ursprünglich allen Zellen des Ektoderms die Fähigkeit zukommt, sich in Linsenfasern, bezw. in linsenfaserartige Elemente umzuwandeln, und dass diese Fähigkeit auch auf gewisse Derivate des Ektoderms — centrales Nervensystem und Augenbecher — übergeht, so wird man sowohl den Thatsachen der normalen Ontogenese (Entwickelung der Linse im Auge und im Parietalorgan), als auch denen der Regeneration (Neubildung der Linse von der Iris, Lentoide der Retina) gerecht«.

In the ontogeny of the Anura, as far as is known, the lens of the eye always arises as in the Urodela from a thickening of the inner layer of ectoderm. KOCHS (9) has shown that in the tadpole of *Rana fusca* a new lens regenerates after the extirpation of the old one, but he was not able to determine its origin. In the experiments made by SPEMANN and by LEWIS the new lens was invariably derived from the ectoderm of the body wall.

Among the embryos with abnormal eyes that were obtained in the course of the present experiments, there were two which seem to furnish evidence that in the Anura, as well as in the Urodela, a lens may occasionally develop from the edge of the optic cup. Fig. 12 is an outline drawing of a part of a section through the head of an embryo that was operated upon the nineteenth of April, 1902, and killed six days later. The embryo was sectioned somewhat obliquely and, therefore, but a very small, pigmented portion of the normal eye is shown on the right of the mid-brain (Fig. 12, *N.E.*); the rest of this eye with its well developed lens and optic nerve lies in sections anterior to the one drawn. The brain of the embryo appears normal and shows not the slightest trace of the operation. Interest in this tadpole centers in the eye on the left side of the body which lies quite close to the mid-brain and 0.316 mm. from the body wall. This optic cup, which is somewhat abnormal, is connected by an optic nerve with the brain in a more anterior section than that shown, the connection being slightly behind that of the normal eye. The structure of this eye is shown more in detail in Fig. 13. The inner layer of the optic cup is composed, as normally, of several rows of cubical cells and the outer layer is formed of a single row of pigmented cells. The lower portion of the cup, as is frequently the case in these abnormal eyes, is not much curved and lies nearly parallel with the wall of the mid-brain. At the upper edge of the cup, where the two layers unite, there is a knob-like structure that bears a striking resemblance to an early stage in the formation of a lens in the eye of *Triton* as figured by Wolff and by Fischel. This rounded projection from the centre of the upper wall of the optic cup extends over four sections of the embryo, and on either side of it the two layers of the optic cup are joined in the normal manner. There is no central cavity in any section of this structure and no indication of a constriction between it and the two layers of the optic cup which run into it. The cells forming this structure are dividing very rapidly and each section shows a large number of karyokinetic figures, many more than I have found in any other part of the eye in any of the embryos that I have studied. In no section of the head of this embryo is there any indication of a thickening of the ectoderm to form a lens, and, judging from the results that have been obtained in the experiments on the eye of *Triton*, I can see no reason why this projection from the upper wall of the optic cup must not be considered as an early stage in the formation of a lens from the tissues of the eye itself.

Fig. 14 shows a portion of a section through the head of another embryo from the same series as that from which Fig. 12 was drawn. The position of the lens in this eye makes it appear highly probable that the lens developed from the edge of the optic cup, although, of course, this cannot be proven. The development of this eye was much more rapid than was that from which Fig. 12 was drawn, and the lens had already become a separate structure at the opening of the optic cup when the embryo was killed. In Fig. 14 the lens is seen to be sharply marked off from the edge of optic cup; but in other section of the eye the flattened surface of the lens lies against the somewhat flattened edge of the optic cup and it might very easily be supposed that the two were connected. Such close contact between the edge of the optic cup and the lens is not usual when the lens is derived from the ectoderm in the normal manner. In this embryo the optic cup lies 0.113 mm. beneath the ectoderm, and its upper edge is so greatly curved that it does not seem as if the lens could possibly have been derived from the ectoderm in the manner shown in Fig. 6. The normal eye on the opposite side of the head is not shown in the figure because the section is somewhat oblique and also because the eye on the injured side lies considerably behind that on the opposite side of the body. The effects of the operation are further shown in this embryo in the formation of the brain wall which is very much thinner than normal on the injured side. The structure of the abnormal eye is shown under higher magnification in Fig. 15. The cells of both layers of the optic cup appear normal and the shape of the cup itself is not as abnormal as is the one shown in Fig. 13. There can be no question but that the rounded structure in the opening of the optic cup is a lens which has a central cavity rather large in proportion to the size of the lens. The lens is somewhat smaller than the one connected with the normal eye on the uninjured side of the body as it measures but 0.060 mm. in diameter while the one connected with the normal eye measures 0.107 mm. It is much to be regretted that transitional stages between the lenses represented in Figs. 13 and 15 could not be obtained.

While it seems to me very probable, from the two cases described above, that the lens in the eye of the frog embryo is capable, under certain conditions, of developing from the edge of the optic cup, I do not wish to state positively that such is the case. It is conceivable, of course, that such a knob-like thickening at the upper edge of the optic cup as that shown in Fig. 12, would never have formed a normal lens, and also that the lens shown in Fig. 14 was derived from the ectoderm, although I can find no indication whatever that such was its origin. Taking the evidence from these two embryos alone it seems to me highly probable that, under certain conditions, the upper edge of the optic cup is capable of beginning the formation of a lens. Whether such a lens would continue to develop and eventually become functional remains to be determined.

IX. Summary.

1) If an entire eye is removed from an embryo of *Rana palustris*, the tissues of the head are incapable of regenerating a new one.

2) There is no development of an eye if all of the eye-forming region of the fore-brain is destroyed before the medullary folds have closed.

3) Destroying any part of the fore-brain except the eye-forming region does not interfer with the normal development of the eye.

4) The eye-forming tissue of the fore-brain appears to have the power of self-differentiation as it will develop into a more or less normal optic cup when removed from its connection with the rest of the brain and without the presence of a lens.

5) Any ectoderm of the head appears to have the power to produce a lens provided it receives the proper stimulus.

6) Contact between the optic cup and the ectoderm is not necessarily the stimulus that leads to the development of a lens, as a lens can be formed from the ectoderm when the optic cup is some distance beneath the surface of the body.

7) A lens-like structure may be formed from the ectoderm when there is no optic cup on the same side of the head. The process appears to be a self-differentiation of lens-forming tissue of the ectoderm.

8) It seems probably that, under certain conditions, a lens can develop from the upper edge of the optic cup as is the case in the regeneration of the lens in the eye of *Triton*.

Bryn Mawr College, Bryn Mawr, Pa., Nov. 11, 1904.

Zusammenfassung.

1) Beraubt man einen Embryo von *Rana palustris* eines ganzen Auges, so sind die Gewebe des Kopfes nicht imstande, ein neues zu bilden.

2) Es kommt nicht zur Entwicklung eines Auges, wenn der gesamte augenbildende Bezirk des Vorderhirns vor dem Schluß der Medullarwülste zertört wird.

3) Die Zerstörung des Vorderhirns bis auf den augenbildenden Bezirk hindert die normale Entwicklung des Auges nicht.

4) Das augenbildende Gewebe des Vorderhirns scheint die Fähigkeit der Selbstdifferenzierung zu besitzen, da es auch außer Zusammenhang mit dem übrigen Gehirn und ohne das Vorhandensein einer Linse sich zu einem mehr oder weniger normalen Augenbecher entwickeln kann.

5) Den spezifischen Reiz vorausgesetzt scheint jede Partie des Kopfectoderms die Fähigkeit der Linsenentwicklung zu besitzen.

6) Die Berührung des Augenbechers mit dem Ectoderm ist nicht unumgänglich notwendig als Reiz für die Linsenentwicklung, da eine Linse auch entstehen kann, wenn der Augenbecher noch in einiger Entfernung unter der Körperoberfläche liegt.

7) Eine linsenähnliche Struktur kann vom Ektoderm hervorgebracht werden, ohne daß ein Augenbecher auf derselben Seite existiert. Der Prozeß scheint eine Selbstdifferenzierung von linsenbildendem Gewebe aus dem Ectoderm zu sein.

8) Es erscheint möglich, daß sich unter gewissen Bedingungen eine Linse aus dem oberen Rande des Augenbechers zu entwickeln vermag, wie dies bei der Linsenregeneration von *Triton* der Fall ist.

Literature.

1) Barfurth, D., und Dragendorff, O., Versuche über Regeneration des Auges und der Linse beim Hühnerembryo. Verhandl. d. Anat. Gesellsch. 1902.
2) Blumenbach, Specimen physiologiae comparatae inter animantia calidi et frigidi sanguinis. Commentationes soc. reg. scient. Gottingensis. Bd. VIII. 1787.
3) Bonnet, C., Oeuvres d'Histoire naturelle et de Philosophie. T. V. Neuchatel 1781.
4) Colucci, V. S., Sulla regenerazione parziale dell' occhio nei Tritoni. Memorie della R. Accad. delle Scienze dell' Ist. di Bologna (Ser. V.) I. 1891.
5) Fischel, A., Über die Regeneration der Linse. Anatom. Anz. Bd. XIV. 1898.
6) —— Über die Regeneration der Linse. Anat. Hefte. Herausgegeben von Merkel und Bonnet. Heft 44. 1900.
7) —— Weitere Mittheilungen über die Regeneration der Linse. Arch. f. Entw.-Mech. Bd. XV. 1902.
8) Herbst, C., Formative Reize in der tierischen Ontogenese. Leipzig 1901.
9) Kochs, W., Versuche über die Regeneration von Organen bei Amphibien. Arch. f. mikr. Anat. Bd. XLIX. 1897.
10) Lewis, W. H., Experimental Studies on the Development of the Eye in Amphibia. I. On the Origin of the Lens. The Amer. Journ. of Anatomy. Vol. III. 1904.
11) Mencl, E., Ein Fall von beiderseitiger Augenlinsenausbildung während der Abwesenheit von Augenblasen. Arch. f. Entw.-Mech. Bd. XVI. 1903.
12) Müller, E., Über die Regeneration der Augenlinse nach Exstirpation derselben bei Triton. Arch. f. mikr. Anat. Bd. XLVII. 1896.
13) Philippeaux, J. M., Note sur la production de l'oeil chez la Salamandre aquatique. Gazette médicale de Paris. T. 2. 1880.
14) Rabl, C., Über den Bau und die Entwicklung der Linse. Zeitschr. f. wiss. Zool. Bd. LXIII. 1898.
15) Schaper, A., Experimentelle Studien an Amphibienlarven. Erste Mittheilung: Haben künstlich angelegte Defekte des Centralnervensystems oder die vollständige Elimination derselben einen nachweisbaren Einfluß auf die Entwickelung des Gesammtorganismus junger Froschlarven? Arch. f. Entw.-Mech. Bd. VI. 1898.

16) Schaper, A., Über einige Fälle atypischer Linsenentwickelung unter abnormen Bedingungen. Anatom. Anz. Bd. XXIV. 1904.

17) Schimkewitsch, W., Über den atavistischen Charakter der Linsenregeneration bei Amphibien. Anatom. Anz. Bd. XXI. 1902.

18) Spemann, H., Über Correlation in der Entwickelung des Auges. Verhandl. d. Anat. Gesellsch. 1901.

19) —— Über Linsenbildung bei defekter Augenblase. Anatom. Anz. Bd. XXIII. 1903.

20) Wolff, G., Bemerkungen zum Darwinismus mit einem experimentellen Beitrag zur Physiologie der Entwickelung. Biol. Centralbl. Bd. XIV. 1894.

21) —— Entwickelungsphysiologische Studien. I. Die Regeneration der Urodelenlinse. Arch. f. Entw.-Mech. Bd. I. 1895.

22) —— Entwickelungsphysiologische Studien. II. Weitere Mittheilungen zur Regeneration der Urodelenlinse. Arch. f. Entw.-Mech. Bd. XII. 1901.

Explanation of Figures.

Plate VI.

All figures were outlined with the aid of a camera lucida. Figs. 1, 2, 5, 7, 9, 12, and 14 were drawn under a Zeiss obj. AA, oc. 4; the other figures were drawn under a Zeiss obj. D, oc. 4.

In lettering the figures the following abbreviations are used: *F.B.* fore-brain; *M.B.* mid-brain; *L.* lens; *N.E.* normal eye; *Ph.* pharynx; *S.* sucker; *O.C.* optic cup.

Fig. 1. Section through the head of an embryo killed four days after the puncture of one side of the fore-brain. An optic cup has developed between the brain and the upper wall of the pharynx.

Fig. 2. Part of a section through the head of an embryo killed four days after the operation. A small optic cup, but no lens, has developed on the injured side of the body.

Fig. 3. Part of a section through the head of an embryo killed six days after the operation. The optic cup that has formed on the injured side of the head is in direct contact with the inner ectoderm but no lens has developed.

Fig. 4. Part of a section showing the abnormal optic cup in an embryo killed five days after the puncture of the fore-brain.

Fig. 5. Outline drawing of a part of a section through the head of an embryo killed six days after the operation. The optic cup is some distance from the ectoderm, a lens is forming in an unusual manner.

Fig. 6. Enlarged drawing of the abnormal eye shown in Fig. 5.

Fig. 7. Section through the head of an embryo killed six after the puncture of the fore-brain. A lens is forming from the ectoderm opposite the lower edge of the optic cup.

Fig. 8. Part of a section through the head of an embryo belonging to the same series as that from which Fig. 7 was drawn. All of the inner layer of ectoderm opposite the optic cup is thickening to form a lens.

Fig. 9. Outline drawing of a part of a section through the head of an embryo killed three days after the puncture of the fore-brain. A lens-like structure is forming from the inner layer of ectoderm without the presence of an optic cup.

Fig. 10. Enlarged drawing of the lens-like structure shown in outline in Fig. 9.

Fig. 11. Enlarged drawing of another, slightly larger, lens-like structure that developed from the ectoderm without the presence of an optic cup.

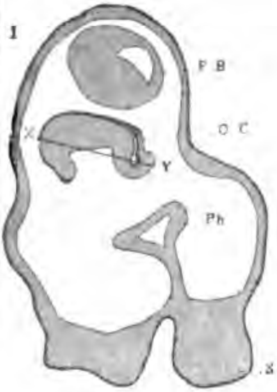
Fig. 12. Part of a section of an embryo killed six days after the operation. There is a knob-like thickening at the upper edge of the optic cup which may be an early stage in the formation of a lens.

Fig. 13. Enlarged drawing of the optic cup shown in outline in Fig. 12.

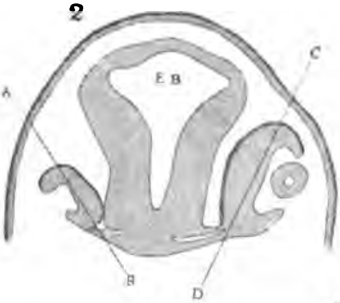
Fig. 14. Part of a section through the head of an embryo belonging to the same series as that from which Fig. 12 was drawn. A lens is present which presumable developed from the edge of the optic cup.

Fig. 15. Enlarged drawing of the optic cup and lens shown in outline in Fig. 14.

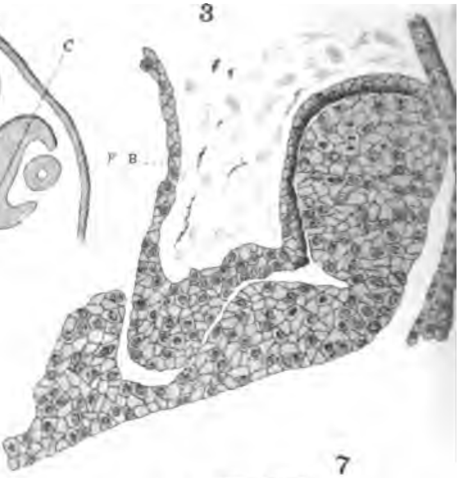
1



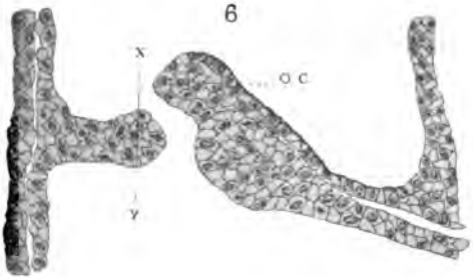
2



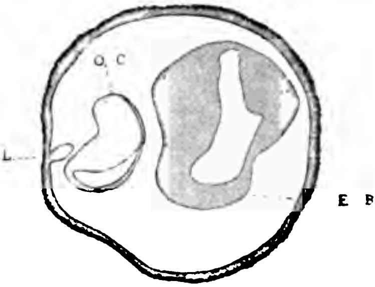
3



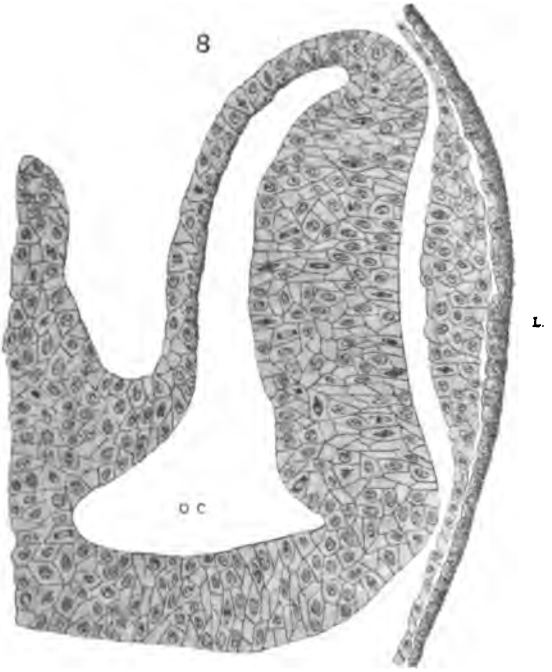
6



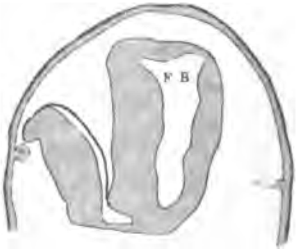
7



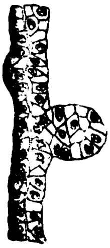
8



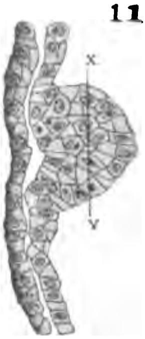
9



10

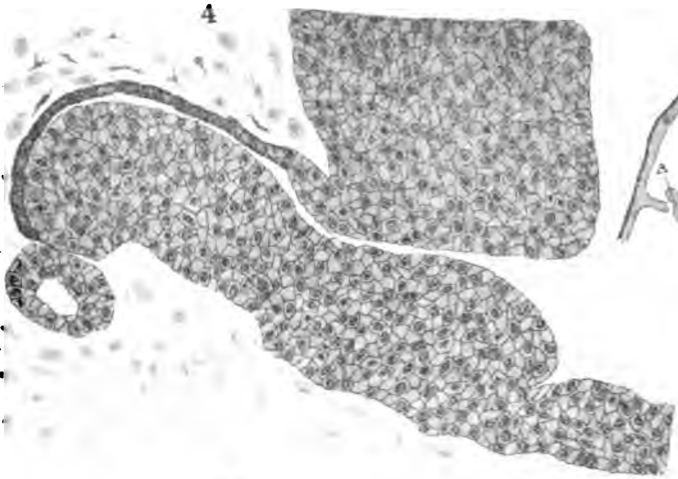


11

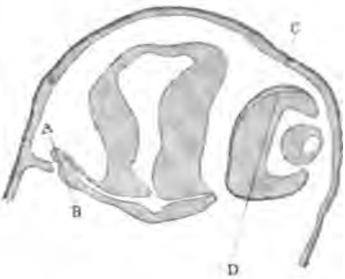


Verlag v Wilhe

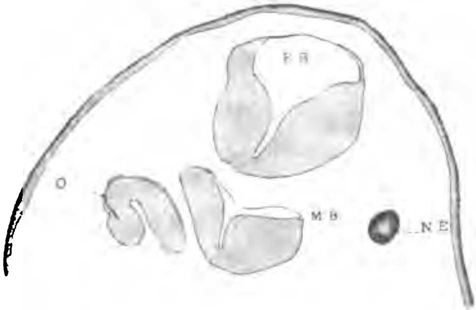
4



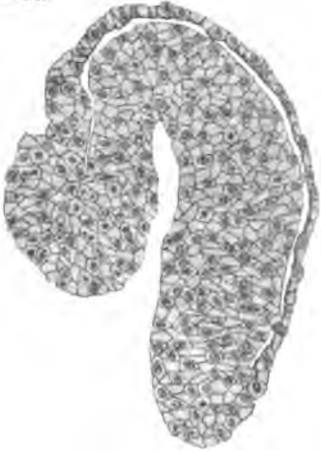
5



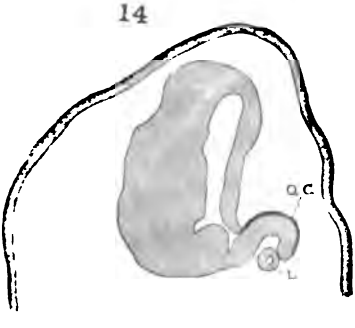
12



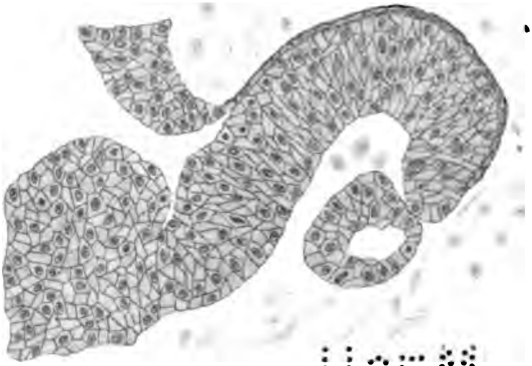
13



14



15



UofM

lmann in Leipzig

Photolithogr. v. H. F. Jütte, Leipzig.

2858 tri.

BRYN MAWR COLLEGE MONOGRAPHS

REPRINT SERIES, Vol. V.

CONTRIBUTIONS FROM THE BIOLOGICAL LABORATORY

BRYN MAWR, PENNA., U. S. A.
April 1905

ANNOUNCEMENT

The Bryn Mawr College Monographs will be issued in two series; the first, the **Monograph Series,** containing articles that appear here for the first time; the second, the **Reprint Series,** containing reprints of articles that have appeared in other journals.

It is proposed to publish these monographs in separate numbers at irregular intervals as material is accumulated. The numbers will be combined into volumes of about 500 pages.

The monographs are edited by a committee of the Faculty of Bryn Mawr College, consisting at present of President M. Carey Thomas, **ex-officio,** Professor Elmer P. Kohler (chairman), Professor Lindley Miller Keasbey, and Professor David Irons.